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The University of Osaka

Doctoral Dissertation

**Investigating the effect of modifying  
tempe's production process to  
metabolome and sensory profile**

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June 2022

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Osaka University

## List of Abbreviations

*(in alphabetical order)*

|       |                                              |
|-------|----------------------------------------------|
| ANOVA | Analysis of variance                         |
| C     | Control tempe                                |
| Cr    | Crushed tempe                                |
| F     | Familiar panelist                            |
| GC    | Gas chromatography                           |
| MS    | Mass Spectrometry                            |
| MSI   | Metabolomics Standards Initiative            |
| MSTFA | N-Trimethylsilyl-N-methyl trifluoroacetamide |
| NF    | Non-familiar panelist                        |
| OPLS  | Orthogonal Projection to Latent Structure    |
| PCA   | Principal Component Analysis                 |
| RMSEE | Root-Mean-Square-Error of Estimation         |
| RATA  | Rate-All-That-Apply                          |
| VIP   | Variable Importance in Projection            |
| V     | Vinegar tempe                                |
| Vc    | Vinegar Crushed tempe                        |

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# Chapter 1

## General introduction

### 1.1 Tempe as a traditional fermented food

Tempe (also written as tempeh) is a traditional fermented food from Indonesia. It is made by inoculating *Rhizopus oligosporus* mold into soybeans. During fermentation, the mold will form a mycelium that will envelop the soybeans. Fermentation is considered complete when the mycelium has a firm texture and had fully enveloped the soybeans. This kind of fermentation is solid-state fermentation. Tempe is high protein plant-based food and had a firm texture due to the mycelium formation. Because of its low cost, tempe had been regarded as a poor's man meat (Malav et al. 2015). Nowadays, people in Southeast Asia and especially Indonesia still consumed tempe as part of their dietary lifestyle. Tempe has regional variety even within Indonesia (Kadar et al. 2020).

Traditionally, soybeans were made by packaging the soybeans with leaves (Ahnan-Winarno et al. 2021). The mycelium will grow naturally after a day of packaging. Based on previous research, *Rhizopus oligosporus* had been suggested to be originated from hibiscus leaves that were common in southeast Asia (Ogawa, Tokumasu, and Tubaki 2004). Nowadays, The Indonesian Institute of Sciences (LIPI) had commercialized the *Rhizopus oligosporus* as a tempe starter culture. This starter culture product is commercially available globally and had been used in various local and international tempe industries. Tempe making method had also modernised with time, where leaf packaging had been replaced with plastics and fermentation parameters had been defined and controlled for improving quality control.

## 1.2 The process of making tempe

The process of making tempe is based on Figure 1.1. Tempe making process starts with the soaking process. The soaking process can last between 6 hr to 24 hr. The soaking process will enable the imbibition of soybeans. Besides imbibition, natural acidification will occur within the soaking water. The acidification is due to the growth of bacteria especially lactic acid bacteria during the soaking process (Mulyowidarso, Fleet, and Buckle 1989). The acidification can also help to inhibit or retardation the growth of pathogens and/or spoilage-causing microbes that might grow during fermentation (Tunçel and Göktan 1990).

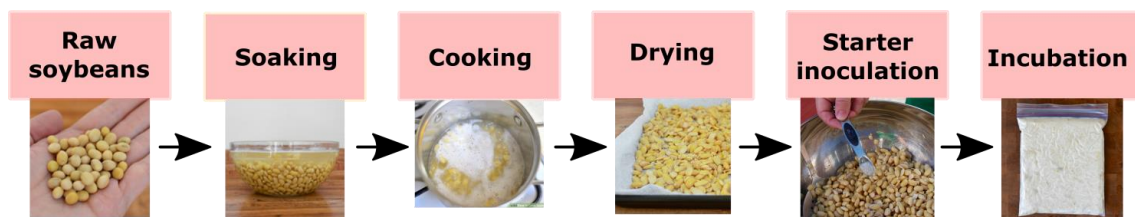


Figure 1.1 The principle of making tempe

The cooking process (also known as the boiling process) was performed after the soaking process. The cooking process removes the soybean's raw flavor as well as eliminates surviving pathogens and spoilage microorganisms (Nout and Kiers 2005). After the cooking step, soybeans are usually dehulled and dried. The dehulling process involves separating the soybean hull from the bean. This process is a requirement for any variation

of tempe making process because tempe standards considered hulls as contaminants (Codex Alimentarius 2017).

The drying step is a process that reduces the water content in the soybean before starter culture inoculation. If the soybean is wet during the starter culture inoculation, the tempe fermentation will fail due to high moisture content. After inoculation, the soybeans are packed into a compact container with limited airflow. The compactness of the container is required to ensure the growth of dense mycelium biomass. Tempe fermentation is usually performed between 25 to 38 °C for 18 to 72 hr (Nout and Kiers 2005). During the solid-state fermentation process, the *Rhizopus* spp. digested protein into amino acids (Ashenafi and Busse 1991), lipids into fatty acids (Ruiz-Terán and David Owens 1996) as well as breaking down isoflavones glycosides into aglycones (Kuligowski et al. 2016). Throughout the tempe making process, the soybean becomes more nutritious by fermenting into becoming tempe.

### **1.3 Challenge of adapting tempe into the diet of new consumers**

Fresh tempe is not eaten raw but were cooked before consumption. As such, tempe is a suitable ingredient for meat alternatives (Malav et al. 2015). Tempe had been made locally by many foreign countries (Figure 1.2). These “localized” tempe usually utilize local raw ingredients to produce their tempe products. Such a production strategy is one of the solution to ensure food security (Leventon and Laudan 2017).



Figure 1.2 Various “localized” tempe from Netherland, Japan, United states, and Spain.

However, tempe as food had difficulty being accepted by new consumers who are not familiar with its nutritional benefit (Kamoshita et al. 2019). One suggestion to increase acceptance among new consumers is by improving its taste and healthiness (Boukid 2021). To achieve this aim, modification of the tempe making process is essential to improve its sensory properties.

#### 1.4 Metabolomics approach

Metabolomics is an analytical technique that can profile metabolites at a given time or condition of a sample. In the context of food processing research, metabolomics can elucidate the metabolite profile of food produced from different processing strategies (Jacobs, van den Berg, and Hall 2020). Metabolomics data can also be used for correlation with other analyses such as sensory evaluation or physical characteristics of food (Putri et al. 2022). The correlation between metabolomics data and another analytical tool can provide rich discussion about the effect or treatment performed on the food. As such, metabolomics is the suitable tool to observe the effect of modifying processing strategies.

Several studies that used metabolomics to study tempe. Metabolomics had been used to classify tempe based on geographical origin (Kadar et al. 2018). Another study of tempe metabolomics focuses on the metabolite profiles of tempe made from different legumes (Rahmawati et al. 2021). However, there was no study that use metabolomics for modifying the processing step of making tempe.

### **1.5 Objective**

Therefore, the objective of this research is to investigate processing strategies that can improve tempe sensory characteristics via a metabolomic approach. To achieve this objective, three strategies were employed. The first strategy is to investigate the metabolite profiles of tempe made from different process. The second strategy is to determine the acceptance of tempe from different process by sensory evaluation. Finally, a correlation of tempe metabolites with sensory evaluation will be conducted to assess the performance of the different process in improving tempe's sensory characteristics.

### **1.6 Thesis outline**

This thesis is composed of 4 chapters. Chapter 1 is the introduction to tempe that includes a general description of tempe, the principle of making tempe and the challenge of adapting tempe into a new consumer's diet.

Chapter 2 described the effects of soaking soybeans in vinegar during tempe making steps. The vinegar soaking processing strategy was evaluated via metabolomics and sensory evaluation.

Chapter 3 extend the vinegar soaking strategy by combining it with soybean crushing steps. The processing strategies in this chapter were evaluated via the determination of selected physical characteristics (texture analysis, color and moisture content analysis), metabolomics and sensory evaluation.

Chapter 4 presents the conclusion of this study and the future perspective that was proposed from this study.

## Chapter 2

### Effects of soaking tempe in vinegar on metabolome and sensory profiles

#### 2.1. Introduction

Tempe (tempeh) is a fermented soybean food that has been consumed in Indonesia since the 16th century. It is usually prepared by fermenting soybeans with *Rhizopus* spp. as the starter mold (Ahnan-Winarno et al. 2021). Tempe is renowned in Indonesia for its high protein, carbohydrate, fat, and dietary fiber contents along with its high digestibility and nutrient absorption (Ahnan-Winarno et al. 2021). Tempe is becoming popular among vegans and vegetarians as a meat substitute and is produced in several countries (Kadar et al. 2018, Nout and Kiers 2005). The consumption of tempe in Indonesia accounts for at least 10% of all consumed protein, which is higher than chicken eggs (1.25%) or meat (3.15%) (Astuti et al. 2000). Majority of tempe manufacturers in Indonesia are home and cottage-scale industry (Kementerian Perindustrian Republik Indonesia 2013). This indicating cultural and economic importance of tempe in Indonesia.

Although tempe is nutritious, the product might not be appealing to first-time consumers, especially outside Indonesia. This is because tempe has an unpleasant fermented smell that will deter new consumers. One way to improve tempe acceptance by first-time consumers is to modify how it is processed. While various tempe production methods in Indonesia have been reported (Astawan, Wresdiyati, and Maknun 2017), the principle of making tempe has remained unchanged. Tempe is usually made by inoculating dehulled soybeans before incubation with *Rhizopus* spp. for 24 to 48 h. Major variations in production methods are the soaking and cooking steps. Soaking is critical for tempe

production because it induces soybean size expansion (Pan and Tangratanaalee 2003) and promotes acidic fermentation (Mulyowidarso, Fleet, and Buckle 1989, Starzynska-Janiszewska, Stodolak, and Mickowska 2014).

The microbial composition of tempe is determined by ecological factors, such as acidification by lactic acid bacteria during soaking (Nout and Kiers 2005). The presence of lactic acid bacteria in the soaking water is considered to be from the soybean itself (Suwanto 2021). Therefore, indigenous bacteria play a role in acidification, resulting in a pH decrease during natural soaking. The pH in the natural process ranges from 4.12 to 4.50, which prevents the growth of contaminating bacteria and contributes to low levels of pathogenic and spoilage microorganisms in the final product (Astuti et al. 2000). Growth of spoilage microorganism during the soaking process will prevent the growth of *Rhizopus oligosporus* and failed the fermentation of tempe (Nout et al. 1987, Tunçel and Göktan 1990). Although lactic acid bacteria can prevent the growth of spoilage microorganisms, it has been found that it does not increase the growth rate of *Rhizopus oligosporus* during tempe fermentation (Magdalena et al. 2022, Feng, Eriksson, and Schnurer 2005). On the other hand, acids such as vinegar are applied to lower the pH and control the microbial composition of tempe in industrial processes (Shurtleff and Aoyagi 2007). Several tempe manufacturers use vinegar (Shurtleff and Aoyagi 2020) although acid is a powerful inhibitor of fungal growth (Nout and Kiers 2005) and vinegar might affect the quality of the fermented product. However, little is known about the monitoring and control of tempe production processes and how they affect tempe products.

Metabolomics focuses on variations in metabolite profiles in various types of biological samples (German, Hammock, and Watkins 2005). Fermented foods such as coffee have

been studied in terms of authentication (Jumhawan et al. 2013) and geographical origin (Putri et al. 2019) using metabolomics. A metabolomic study has also been used in tempe to classify tempe based on geographical origin and the effect of different legume materials (Rahmawati et al. 2021, Kadar et al. 2018). This technique could be applied to this research to determine how adding vinegar during the soaking process affects the metabolomic and sensory profiles of tempe.

This study aimed to define the effects of soaking tempe in vinegar using metabolome analysis, gas chromatography/mass spectrometry (GC/MS), and sensory profiling to provide useful information to the tempe industry. Our results should also provide a viable tempe processing strategy for introducing first-time consumers to tempe.

## **2.2 Materials and Methods**

### **2.2.1 Tempe Sample Preparation**

Raprima brand (Aneka Fermentasi Industri, Bandung, Indonesia) starter culture was used for this study. The Japanese soybeans used for making the tempe are Yukihome variety (grown in Ishikari, Japan). Tempe was washed, soaked, and boiled with mineral water (Suntory, Tokyo, Japan). The type of vinegar used in this study was Kokumotsu vinegar Mizkan Ltd. (Handa, Japan).

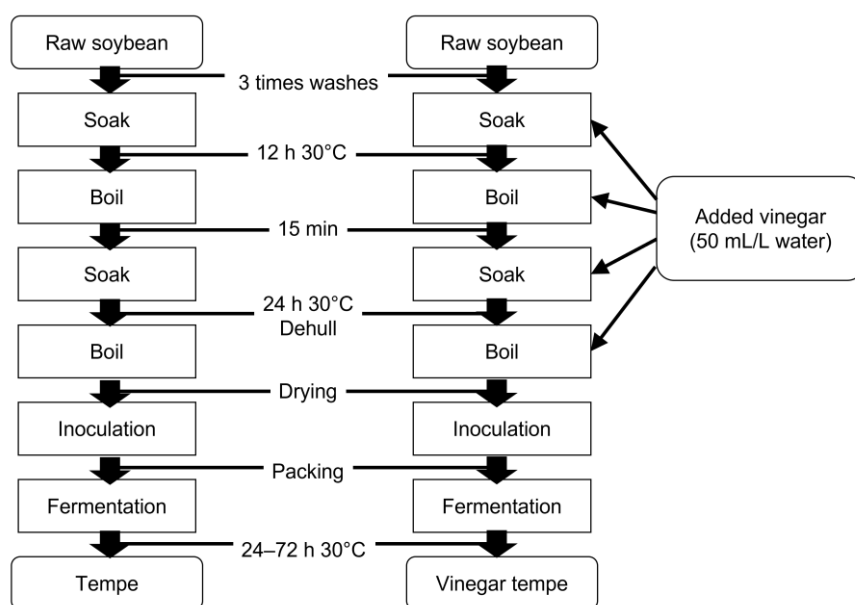


Figure 2.1. Workflow of production of control and vinegar tempe using Rumah Tempe Indonesia (RTI) method

Tempe was processed according to the Rumah Tempe Indonesia (RTI) method (Figure 2.1) (Astawan, Wresdiyati, and Maknun 2017). As for vinegar tempe, the soybeans were soaked and boiled in vinegar (50 mL/L of mineral water). The tempe was packaged, then fermented in an incubator (EYELA, Tokyo, Japan) at 30 °C for 24 to 72 h until the desired texture was achieved. Metabolite changes during fermentation were determined by sampling the tempe for each day until it reached full fermentation (24 and 48 h for control tempe, and 24, 48, and 72 h for vinegar tempe).

## 2.2.2 Metabolite Extraction

Fully fermented tempe were frozen and milled into fine powder and stored in a freezer. A total of 15 mg of lyophilized tempe was mixed and vortexed with 1 mL of a 5:2:2 (v/v/v) ratio of methanol, ultrapure water (both reagents are from Wako Pure Chemical

Industries, Osaka, Japan), chloroform (Kishida Chemical Co. Ltd., Osaka, Japan) and the internal standard ribitol (100 µg/mL). The mixture was centrifuged at 4 °C and 11,740× *g* for 3 min, then 400 µL of supernatant was mixed with ultra-pure water (400 µL) in 1.5 mL microtubes and vortexed to induce separation between the polar and non-polar phases. The mixture was centrifuged for 3 min at 4 °C, 11,740× *g* for 3 min. The aqueous phase (400 µL) was transferred to fresh 1.5 mL microtubes with holed caps. Pooled quality controls (QCs) were prepared by mixing 200 µL of the aqueous phase from all samples. The solvent was evaporated using a Spin Dryer Standard (Taitec Co., Kyoto, Japan) for 2 h at room temperature. The evaporated samples were lyophilized overnight (Taitec Co.).

### 2.2.3 GC/MS Analysis

Samples were derivatized prior to GC/MS injection to analyze the non-volatile metabolites. Lyophilized samples were oximized with 100 µL of methoxyamine hydrochloride (20 mg/mL in pyridine) (Sigma-Aldrich, St. Louis, MO, USA) then shaken at 161 × *g* in an incubator at 30 °C for 90 min. The samples were silylated with 50 µL *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA) (GL Sciences Inc.), then incubated at 37 °C for 30 min at 161× *g*.

Metabolites analysis were conducted using GC/MS-QP2010 instrument (Shimadzu, Kyoto, Japan) equipped with a 0.25 × 30 mm, 0.25-µm Inert Cap 5 MS/NP column (GL Sciences Inc., Tokyo, Japan), and an AOC-20i/s autosampler (Shimadzu, Kyoto, Japan). The data acquisition condition for the instrument is based on a previous study of coffee metabolomics [13]. The reference for retention time is based on standard alkane mixture (C8–C40) that was injected before sample analysis.

#### **2.2.4 GC/MS Data Analysis**

The GC/MS data were first converted into ANDI files (\*.cdf) using GCMS Solution software (Shimadzu, Kyoto, Japan). The converted data files were then processed in MS-DIAL v. 3.70 (Riken, Kanagawa, Japan) where the peaks were detected, the baseline was corrected, and peak retention times were aligned (Tsugawa et al. 2015). Peaks were annotated based on the retention index and MS information in the GL-Science Database. The GL-Science Database is a public metabolite library shared in the MS-DIAL website by RIKEN (<http://prime.psc.riken.jp/compms/msdial/main.html>). The relative intensity of each metabolite was normalized by comparison with ribitol as the internal standard. Data analyses were conducted based on differences and similarities by multivariate analysis using SIMCA-P+ v.13 (Umetrics, Umeå, Sweden) for principal component analysis (PCA). The class for each annotated metabolite was determined using the Human Metabolome Database (<https://hmdb.ca/>).

#### **2.2.5 Sample preparation for Triangle and Rate-All-That-Apply (RATA) Tests**

The fully fermented tempe (control tempe 48 h and vinegar tempe 72 h) were sampled for the sensory evaluation. The tempe samples were cut into cubes, cooked in an air fryer (Innsky, Shenzhen, China) for 5 min at 200 °C and served in plastic cups labeled with random three-digit codes.

### **2.2.6 Triangle Test**

A panel of 25 assessors (average age, 26 y; female, n = 12; male, n = 13 men; Japanese, n = 17; non-Japanese n = 8) were trained on the five-basic taste test prior to actual test. Three tempe samples were simultaneously presented in the triangle test. Among the three, two were the same type, and one was a different type (Sinkinson 2017). The test was conducted twice with two sets of test samples per test, comprising samples A (control) and B (vinegar tempe). The combinations and orders were randomized in each set for each panelist. Only panelists who identified the differences in both test were considered successful in detecting perceptible sensory differences. Figure S4 shows the test questionnaire. The results of the triangle tests were analyzed using  $\alpha$  risk statistics based on the British Standard ISO 4120-2004 (2017, Sinkinson 2017).

### **2.2.7 Rate-All-That-Apply (RATA) Tests**

A panel of 27 assessors (average age, 29.5 y; female, n = 10, male, n = 17; Japanese, n = 19; non-Japanese, n = 8) participated in these tests. During selection, the panelists were questioned about their familiarity with the sensory characteristics of tempe. Regardless of their responses, the panelists were trained prior to actual test to familiarize themselves with the 16 sensory attributes of tempe. Control tempe (A) and vinegar tempe (B) samples were presented along with a questionnaire with a list of 16 sensory attributes. The combinations and order of the samples were randomized (AB and BA) for each panelist, and they rated the intensity of each attribute using a 5-point scale. The 16 sensory attributes were based on previous studies (Kustyawati 2017, Codex Alimentarius 2017, Technical Committee ISO/TC 34 2008, Pujchakarn, Suwonsichon, and Suwonsichon

2016, Lee and Chambers 2007). The important tempe attributes (color, texture, aroma and taste) were initially determined based on the tempe standard (Codex Alimentarius 2017). Afterward, specific sensory attributes were collected from previous published tempe and similar food sample sensory researches (Kustyawati 2017, Pujchakarn, Suwonsichon, and Suwonsichon 2016). 16 sensory attributes were selected based on the familiarity with layman (Lee and Chambers 2007). The standard description of these sensory attributes were based on sensory lexicon standard for used in questionnaire (Technical Committee ISO/TC 34 2008). Figure S5 shows the test questionnaire, and Table S3 describes each of the 16 attributes.

The intensity of the RATA data was found to be non-normally distributed and was analyzed using Mann–Whitney U-tests for statistical comparisons (Meyners, Jaeger, and Ares 2016), and visualized using raincloud plots (Allen et al. 2021) and principal component analysis. Data in the PCA were center-scaled and power transformed using SIMCA-P+ version 13 (Umetrics). Raincloud plots were created using JASP software v. 0.15 (JASP Team 2021).

## **2.3 Results and Discussion**

### **2.3.1 Metabolite profiles of control and vinegar tempe**

The amount of time required to completely ferment control tempe and vinegar tempe differed; the desired texture of control tempe was achieved in 48 h while it takes 72 h for vinegar tempe to achieve the desired tempe texture (Figure S1). Fermentation for vinegar tempe was delayed due to presence of vinegar which inhibits spoilage microorganisms growth (Astuti et al. 2000). Since the metabolites of tempe change according to

pretreatment and duration of fermentation (Cao et al. 2019), samples of control tempe and vinegar tempe collected at 24 and 48 h, and at 24, 48, and 72 h were analyzed by GC/MS. A total of 115 compounds in the tempe samples that were annotated, including 32 unknown metabolites (Table S1). Unknown metabolites are metabolites where the intensity and occurrence of the metabolites passes the peak selection parameter. However, the identification of the metabolites failed to match with any metabolites within the library utilized in this study. The removal of unknown metabolites were not advisable because these metabolites can still be quantified and contribute to the study (Sumner et al. 2007). The processed GC/MS dataset was analyzed using multivariate analysis, and differences between the samples were determined by principal component analysis (PCA).

The results of the PCA showed variations of 61.2% and 15.6% for PC1 and PC2, respectively (Figure 2.2 A). Based on how the samples were grouped, the axis of PC1 shows the progression of fermentation time, and the axis of PC2 shows differences between soaking the control and vinegar tempe as fermentation progressed. The metabolite profiles differed between control tempe fermented to 48 h and vinegar tempe fermented to 72 h.

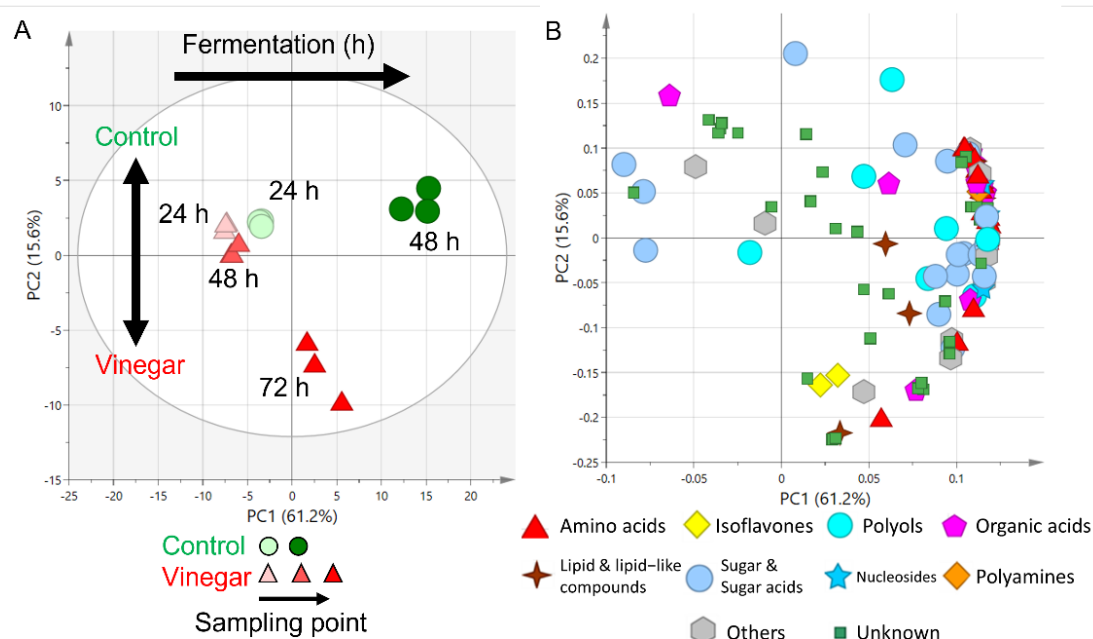


Figure 2.2 Principal component analysis (PCA) of tempe fermented without and with vinegar for 24 48 h, and for 24, 48, and 72 h, respectively. (A) Score plot. Circles and triangles of different colors represent tempe soaked without (control) and with vinegar, sampled at various points (B) Loading plot. Different shapes and colors represent metabolite classes.

Figure 2.2B shows the PCA loading plots. Most metabolites, including those of amino acids, isoflavones, polyols, sugar and sugar acids, nucleosides, polyamines, and organic acids, were positioned on the positive side of PC1. The class of metabolites detected coincided with previous tempe study (Kadar et al. 2020). These results indicated that the amount of these metabolites increased as mycelia grow throughout tempe fermentation, regardless of the soaking conditions. This coincided with previous reports of increased isoflavones (Kameda et al. 2018) and amino acids (Denardi-Souza et al. 2017) during tempe fermentation. The metabolites of fructose, arabitol, n-propylamine, malonic acid, gluconic acid, glucono-1,5-lactone, sorbose, psicose, and tagatose were located on the

negative side of PC1, indicating decreases in their quantities as tempe fermented. Most of these metabolites were from sugars and sugar-alcohols metabolite class. The decrease in sugar-based metabolites is reasonable because they are degraded during the formation of *Rhizopus* sp. mycelia (Rehms and Barz 1995). Table S2 shows the loading values of all detected and annotated metabolites. Tempe soaked without and with vinegar was fermented for 48 and 72 h, respectively, in subsequent sensory analysis, based on the increase of most metabolites.

### **2.3.2. Sensory differences between control and vinegar tempe via triangle tests**

We investigated the sensory differences between control tempe and vinegar tempe using triangle tests (Table 2.1). The objective of the sensory triangle test is to determine the existence of perceptible sensory differences between two products (Sinkinson 2017). The results showed that 15 out of 25 panelists could discriminate control tempe and vinegar tempe. The determination of discrimination is done using probability test called alpha ( $\alpha$ ) risk. Alpha risk is probability of obtaining type 1 error and it can be determined by observing the number of correct answers with the alpha ( $\alpha$ ) risk table which shows the minimum number of correct answers to conclude that there is a perceptible difference between products (Sinkinson 2017). The alpha ( $\alpha$ ) risk table of statistics showed that the sensory difference between tempe fermented with and without vinegar was detectable at an  $\alpha$ -risk level of 0.01 (2017).

The triangle test result was notable because the panelists included individuals who did not regularly consume tempe, and they were able to detect a perceptible sensory difference between the two types of fermented tempe. However, the triangle test does not describe sensory attributes that differ between two samples. Therefore, we continue the

investigation with rate-all-that-apply (RATA) tests in order to know what the sensory differences between control tempe and vinegar tempe.

Table 2.1. Details and results of triangle test.

| Triangle test                                                          |                                             |
|------------------------------------------------------------------------|---------------------------------------------|
| Number of panelists                                                    | 25 (13 male, 12 female)                     |
| Sample                                                                 | 2 × Control tempe (48 h)                    |
|                                                                        | 1 × Vinegar-treated tempe (72 h)            |
| Number of panelists successful distinguishing<br>vinegar-treated tempe | 15                                          |
| Alpha risk study (Differentiation test)                                | Significantly different ( $\alpha = 0.01$ ) |

### 2.3.3. Sensory attributes of control and vinegar tempe using rate-all-that-apply (RATA) tests

Table S3 shows the 16 sensory attributes included in the RATA test. These attributes are based on the categories of color (golden-brown), texture (compact and crispy), aroma (beany, tempe-specific, alcohol, pleasant, yeast, and ammonia), taste (sweet, sour, salty, bitter, umami, astringent), and hedonic (acceptance).

Among the 27 panelists who participated in the RATA tests, 11 panelists were familiar with tempe sensory characteristics, and 16 panelists were not familiar with tempe sensory characteristics. Tables S4 and 2 show the characteristics of the 27 panelists and the descriptive statistics of the sensory attributes in the test. Based on descriptive statistics (Table 2.2), all sensory attributes significantly differed ( $p < 0.05$ , Shapiro–Wilk tests) and were transformed to create a PCA plot, which shows variations of 18.6% and 12.9% in

PC1 and PC2, respectively (Figure 2.3A). The small variation (%) in both principal component axes indicate small perceptible sensory differences based on the 16 attributes. However, the separation of control tempe and vinegar tempe clusters on the negative and positive sides of PC1 showed that most panelists could differentiate the sensory attributes of control and vinegar tempe.

Table 2.2 Descriptive statistics of sensory attributes in RATA tests.

| <b>Sensory Attributes</b> | <b>Tempe Types</b> | <b>Median</b> | <b>Mean</b> | <b>Std. Deviation</b> | <b>p-Value of Shapiro–Wilk</b> | <b>p-Value of Mann–Whitney U</b> |
|---------------------------|--------------------|---------------|-------------|-----------------------|--------------------------------|----------------------------------|
| Golden-brown              | Control            | 3             | 3.37        | 0.839                 | 0.004                          | 0.009                            |
|                           | Vinegar            | 3             | 2.741       | 0.764                 | 0.001                          |                                  |
| Compact                   | Control            | 3             | 3.148       | 0.907                 | <0.001                         | 0.503                            |
|                           | Vinegar            | 3             | 3.296       | 0.869                 | 0.003                          |                                  |
| Crispy                    | Control            | 3             | 2.481       | 0.975                 | 0.006                          | 0.207                            |
|                           | Vinegar            | 2             | 2.148       | 0.864                 | 0.002                          |                                  |
| Beany                     | Control            | 3             | 3.037       | 1.16                  | 0.032                          | 0.666                            |
|                           | Vinegar            | 3             | 3.148       | 0.949                 | 0.009                          |                                  |
| Specific tempe            | Control            | 3             | 3.222       | 1.086                 | 0.027                          | 0.359                            |
|                           | Vinegar            | 4             | 3.481       | 0.975                 | 0.006                          |                                  |
| Alcohol                   | Control            | 2             | 1.815       | 0.921                 | <0.001                         | 0.316                            |
|                           | Vinegar            | 1             | 1.556       | 0.751                 | <0.001                         |                                  |
| Pleasant                  | Control            | 2             | 2.148       | 0.989                 | <0.001                         | 0.003                            |
|                           | Vinegar            | 3             | 3           | 1.109                 | 0.036                          |                                  |
| Yeast                     | Control            | 3             | 2.963       | 1.315                 | 0.024                          | 0.228                            |
|                           | Vinegar            | 3             | 2.519       | 1.189                 | 0.012                          |                                  |
| Ammonia                   | Control            | 2             | 2.593       | 1.526                 | <0.001                         | 0.041                            |
|                           | Vinegar            | 1             | 1.741       | 1.023                 | <0.001                         |                                  |
| Sweet                     | Control            | 2             | 1.852       | 0.77                  | <0.001                         | 0.227                            |
|                           | Vinegar            | 2             | 2.333       | 1.271                 | 0.002                          |                                  |
| Sour                      | Control            | 2             | 2.185       | 1.145                 | <0.001                         | 0.063                            |
|                           | Vinegar            | 1             | 1.593       | 0.694                 | <0.001                         |                                  |
| Saltiness                 | Control            | 3             | 2.741       | 1.023                 | 0.002                          | 0.006                            |
|                           | Vinegar            | 2             | 1.926       | 0.958                 | <0.001                         |                                  |

(Continued to next page)

(Continued from previous page)

|               |         |   |       |       |        |       |
|---------------|---------|---|-------|-------|--------|-------|
| Bitter        | Control | 2 | 2.296 | 1.031 | 0.004  | 0.392 |
|               | Vinegar | 2 | 2.074 | 1.072 | <0.001 |       |
| Umami         | Control | 3 | 2.963 | 0.98  | 0.024  | 0.453 |
|               | Vinegar | 3 | 2.704 | 1.068 | 0.002  |       |
| Astringent    | Control | 2 | 2.259 | 1.13  | 0.001  | 0.231 |
|               | Vinegar | 2 | 1.926 | 1.072 | <0.001 |       |
| Acceptability | Control | 3 | 2.778 | 0.934 | 0.017  | 0.026 |
|               | Vinegar | 4 | 3.407 | 1.083 | 0.02   |       |

SD, standard deviation. Mann–Whitney U tests.

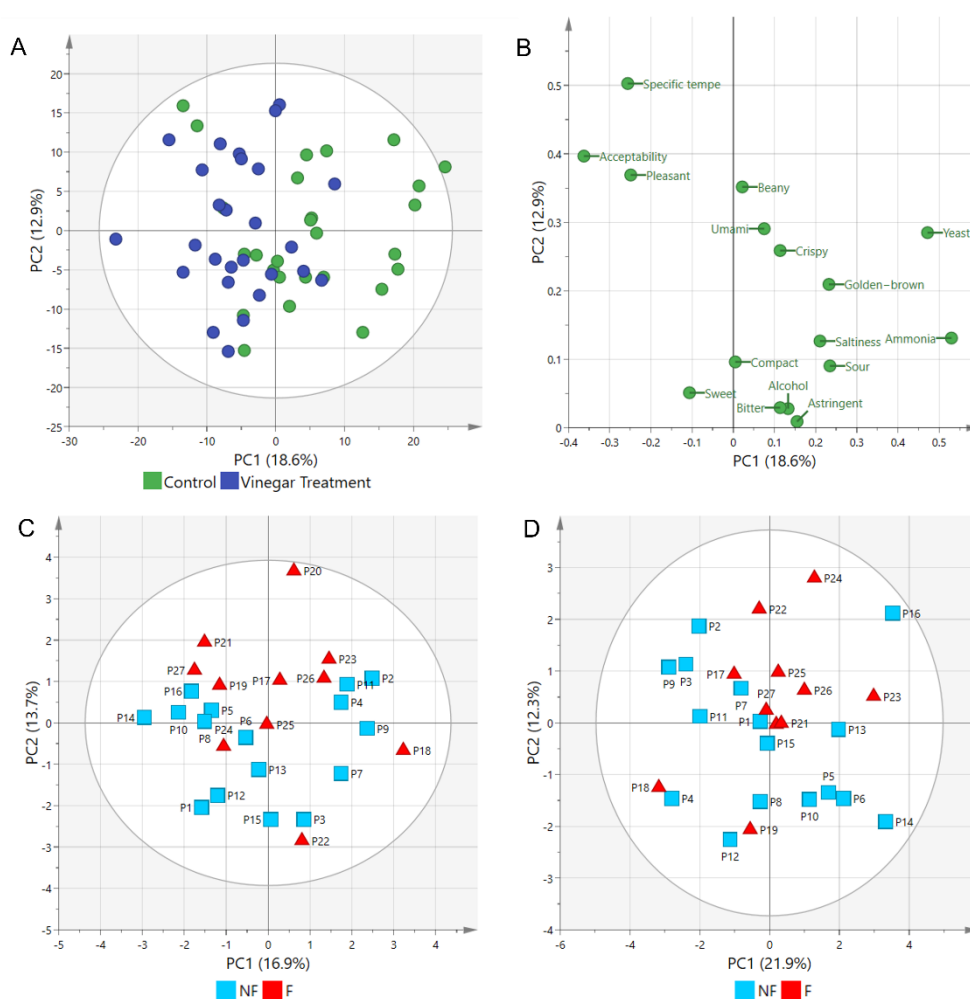


Figure 2.3. Principal component analysis (PCA) of rate-all-that-apply (RATA) tests of two types of tempe and panelist performance. (A) Score plot. (B) Loading plot. Panelist performance was based on familiarity with tempe sensory characteristics before RATA tests. Score plots of panelist performance for control tempe (C) and vinegar tempe (D).

Figure 2.3B shows the PCA loading plot of the RATA test. The positions of the 16 sensory attributes indicated that most of them have higher intensity in control tempe. Only tempe-specific smell, pleasant smell, sweet taste, and acceptability were higher in vinegar tempe. Another notable result from the RATA test was the grouping of the panelists based on their familiarity with tempe sensory characteristics. Figure 2.3C and D shows PCA plots of the RATA panelists for the two types of tempe. Most of the panelists who were familiar with the sensory characteristics of tempe were grouped together on the positive side of PC2. This trend was similar for both control (Figure 2.3C) and vinegar tempe (Figure 2.3D). The group differences between the panelists can explain the non-normal distribution of the RATA results. The results also indicated that preferences differed between panelists who were newly introduced to tempe in this study, and those who were already familiar with tempe sensory characteristics.

The statistical analysis of the 16 sensory attributes showed that only golden-brown color, saltiness, ammonia smell, pleasant smell, and acceptability attributes (Table 2) achieved statistical significance ( $p < 0.05$ ). All five significant sensory attributes were analyzed using raincloud plots (Figure 2.4), as these plots can visualize RATA panelist choices in terms of raw data, box plots, and probability density (Allen et al. 2021). Three of the sensory attributes, namely saltiness (Figure 2.4A), golden-brown (Figure 2.4B), and ammonia (Figure 2.4C), were significantly higher ( $p < 0.05$ ) in the control tempe (Table 2.2). This is clearly shown in box and dot plots, where more panelists scored these three significant sensory characteristics higher in the control tempe. The panelists were questioned about their preferences for colors in a range from light to darker golden-brown, whether they associated the taste of tempe with sodium chloride (salty), and whether the tempe provoked a sharp sensation of ammonia smell. The results of the raincloud plot

showed that most panelists rated the control tempe as salty, golden-brown, and ammonia smell.

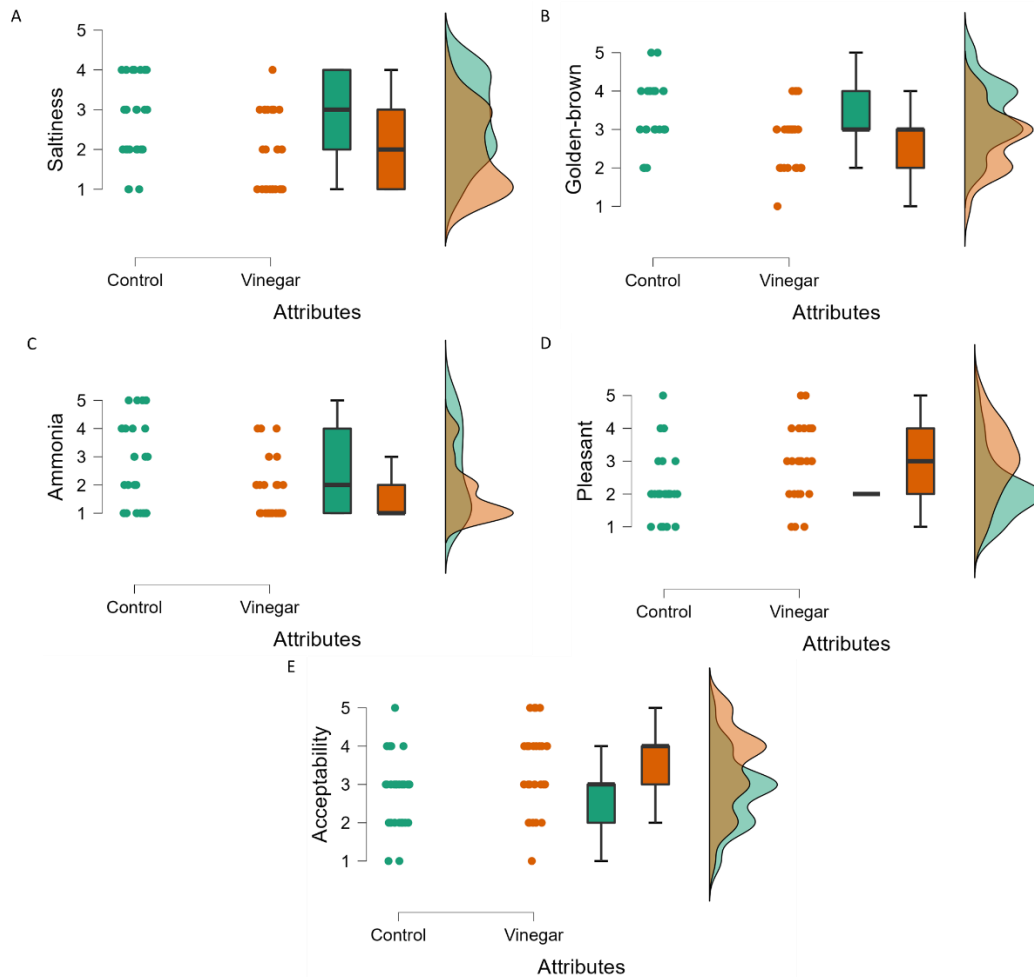


Figure 2.4. Raincloud plots for five significantly different sensory attributes ( $p < 0.05$ ). (A) Saltiness, (B) golden-brown color, (C) ammonia smell, (D) pleasantness and (E) acceptability. Raincloud plots comprise a combination of dot, box and distribution plots.

The non-normal distribution of the panelist results was due to the 11 and 16 panelists who were respectively familiar and unfamiliar with tempe sensory attributes and judged the attributes differently. As most panelists were judging tempe sensory characteristics for the first time in this study, we deduced that they found the tempe fermented with vinegar

to be less salty, lighter in color, and smelled less of ammonia than the control, whereas those familiar with tempe sensory characteristics had different opinions.

In addition to these three significant sensory characteristics, pleasant smell and hedonic acceptance attributes were also statistically significant. The pleasant smell attributes asked the panelists about which types of tempe have aroma characteristics with positive connotations. The acceptance attribute describes the acceptance of a tempe based on overall value, concurrent with all attributes. The pleasant smell (Figure 2.4D) and acceptance attributes (Figure 2.4E) were significantly higher ( $p < 0.05$ ) in the tempe fermented with vinegar (Table 2.2). A boxplot for pleasant smell in control tempe was not created due to similar values for the 25th and 75th quartiles of the data. However, the dot plot and distribution plot were sufficient to visualize differences in panelist choice between the two types of tempe. The dot plot and skewed distribution plot showed that most panelists felt that the smell was more pleasant for vinegar tempe than the control (Figure 2.4D). This result is antagonistic to the ammonia smell (Figure 2.4C), as most panelists felt that tempe fermented with vinegar smelled less of ammonia.

The raincloud plots (Figure 2.4E) show that most panelists preferred the vinegar tempe than control tempe, which was reasonable because vinegar tempe had lesser salty taste, lesser ammonia smell, and a more pleasant smell. The results also revealed that the panelists who were new to tempe preferred lighter color over darker, golden-brown tempe. These findings indicated that soaking soybeans in 50 mL/L of vinegar during tempe processing is a viable processing strategy for introducing tempe to people without knowledge of its sensory characteristics. Figure S2 shows raincloud plots for all other sensory attributes.

#### **2.3.4. Metabolite profile of control tempe and vinegar tempe**

The different intensity of sensory attributes among the RATA panelists regardless of tempe familiarity is due to the metabolite profile of the control and tempe soaked in vinegar. The results of the PCA of tempe soaked without and with vinegar before fermentation showed variations of 67.6% and 13.9% for PC1 and PC2, respectively (Figure 2.5A). The large variance and clear differences in samples group position were able to visualize the differences between the metabolomic profiles of control tempe and vinegar tempe.

The loading plot shows that both types of tempe contain different amounts of various metabolite classes (Figure 2.5B). Most metabolites were positioned on the positive side of PC1, indicating that the control tempe contained more amino acids, organic acids, polyols, sugars, nucleosides, and other classes compared to vinegar tempe (Figure S3). Polyols and amino acids accumulate during tempe fermentation (Ruijter, Visser, and Rinzema 2004, Ahnan-Winarno et al. 2021). In contrast, the metabolites on the negative side of PC1 represent vinegar tempe, and comprised of raffinose, glutamine, 2-hydroxypyridine, genistein, fructose-6-phosphate, oxalate, daidzein, phosphate, sucrose, ornithine, myristic acid, N- $\alpha$ -acetyl ornithine, and sorbose. Raffinose and sucrose had been found to be degraded during tempe fermentation (Rehms and Barz 1995). However, our results showed that soaking in vinegar decreased the degradation rate of various sugar metabolites. Similarly, the retention of daidzein and genistein in soybeans is affected by the type of soaking water in which the soybeans is soaked (Tan, Chang, and Zhang 2016). Our results showed that although vinegar tempe had fewer amino acid metabolites, it had more daidzein and genistein. Table S4 and Figure S3 show the loading values of all metabolites and a bar graph of all annotated metabolites, respectively.

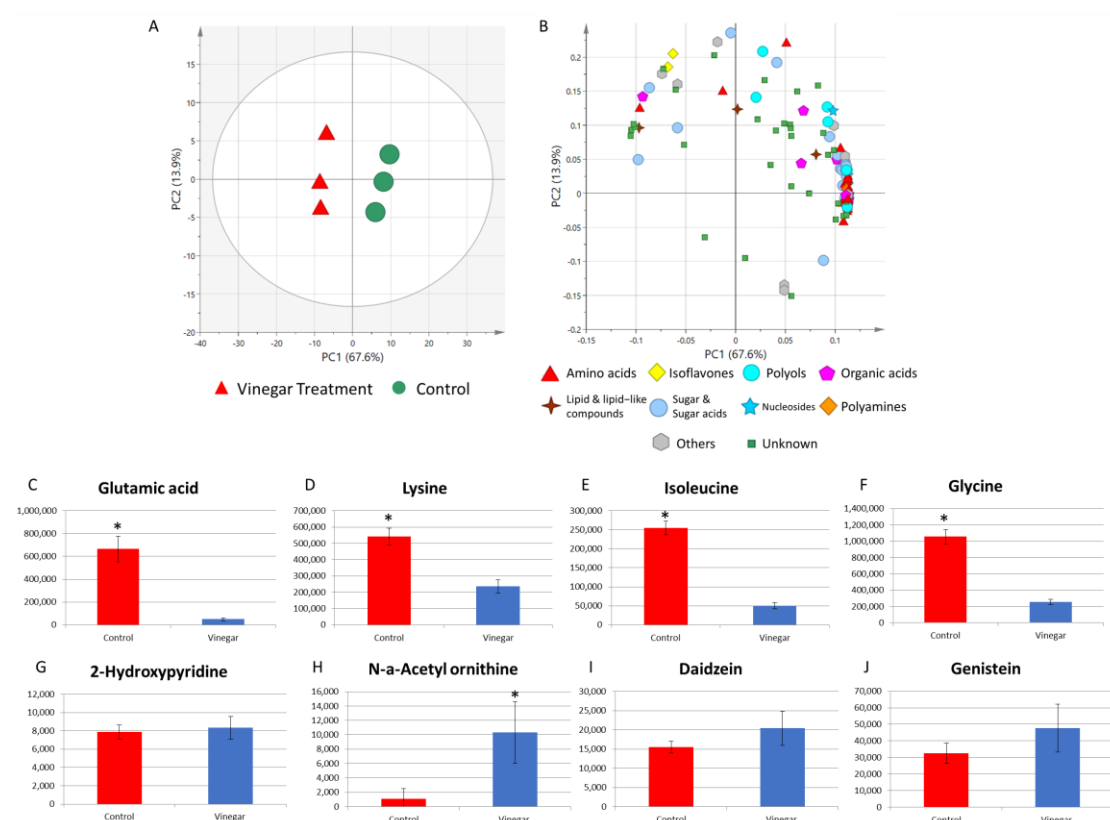


Figure 2.5 Principal component analysis of control tempe and vinegar tempe and bar graph of selected metabolites. (A) Score and (B) loading plots of control tempe and vinegar tempe. Bar graph shows relative intensity of (C) glutamic acid, (D) lysine, (E) isoleucine, (F) glycine, (G) 2-hydroxypyridine, (H) N- $\alpha$ -acetylornithine, (I) daidzein, and (J) genistein. The symbol “\*” denotes significantly different ( $p < 0.05$ ) in Student’s t-test.

The metabolite profiles can explain the sensory differences between the two types of tempe; the amino acid metabolite class differed the most between the two groups (Figure 2.5B). One example is glutamic acid ( $p < 0.05$ ) (Figure 2.5C), which is partly responsible for the umami taste (Ninomiya 2015) and saltiness in other fermented foods (Schindler et al. 2011). However, the RATA panelists found no significant differences in umami sensory attributes between the two types of tempe (Figure S2), whereas the high relative intensity of glutamic acid coincided with the intensity of high saltiness in the control

tempe. Control tempe contained more glutamic acid, lysine, isoleucine, and glycine (Figure 2.5D–F). These three amino acids are highly reactive toward Maillard reaction (Hemmler et al. 2018). The high relative intensity of free amino acid reactions with reducing sugars in the control tempe would result in a deeper golden-brown color (Starowicz and Zieliński 2019) as perceived by the panelists.

In addition to the high intensity of amino acids in control tempe, the dominant metabolites can also be associated with sensory characteristics in vinegar tempe. The metabolite 2-hydroxypyridine (Figure 2.5G) is associated with roasted smell (Maga 1981). Similarly, N- $\alpha$ -acetyl ornithine (Figure 2.5H), which was significantly more abundant ( $p < 0.05$ ) in the vinegar tempe, is associated with the taste of cooked meat (Sawano et al. 2020). Although the amount of 2-hydroxypyridine was not significantly higher in vinegar tempe than in control tempe, the combination with significantly higher content of N- $\alpha$ -acetyl ornithine might have influenced the RATA panelists to rate the hedonic acceptability sensory attributes vinegar tempe more highly. The two isoflavones; daidzein (Figure 2.5I) and genistein (Figure 2.5J) can also elicit a bitter taste (Roland et al. 2011). However, our RATA panelist results felt that there were no differences in bitter taste between the two types of tempe (Figure S2). The intensity of daidzein and genistein were also not significantly higher in vinegar tempe and control tempe. However, the RATA results showed that vinegar can elicit a similar level of bitter taste even though the intensity of bitter-tasting amino acids such as isoleucine are lower in control tempe (Stoeger et al. 2020). Considering all the significant sensory attributes, the decreased ammonia smell and the salty taste combined with a pleasant smell and a lighter golden-brown color led most panelists to accept the vinegar tempe more than the control tempe.

## 2.4 Conclusion

In summary, metabolite profiling using GC/MS showed that vinegar soaking altered the metabolite production during tempe fermentation, which led to different metabolite profiles in the final products. The different metabolite profiles of vinegar tempe and control tempe were validated using triangle tests, which reveal that there are sensory differences, and RATA tests, which specifies distinctive sensory attributes between the two types of tempe. The RATA sensory test results showed that preferences differed between panelists who were familiar and unfamiliar with the sensory characteristics of tempe. Among the 16 sensory attributes tested, 5 sensory attributes; golden-brown color, ammonia smell, pleasant smell, salty taste, and acceptance significantly differed ( $p < 0.05$ ) between the two types of tempe. The non-normal distribution of the RATA results indicated that most panelists who were new to the sensory characteristics of tempe preferred vinegar tempe over control tempe. The metabolomic findings of the two types of tempe indicated that high intensity of specific amino acid metabolites in control tempe were responsible for the ammonia smell, salty taste, and golden-brown color. In contrast, vinegar tempe contained more metabolites associated with the taste of roasted and cooked meat. The panelists indicated that vinegar tempe smelled more pleasant than control tempe, probably because of the differences in metabolite profiles. Thus, the panelist rates of acceptance of sensory attributes were higher for the vinegar tempe. We found that soaking in vinegar process could be a feasible strategy for introducing tempe to new consumers with no prior knowledge of its sensory characteristics. To the best of our knowledge, this is the first study to reveal changes in the metabolite profiles and sensory attributes of vinegar tempe.

## **Chapter 3**

### **Effect of physical crushing and vinegar soaking to metabolome, physical characteristics, and sensory profiles**

#### **3.1 Introduction**

In the previous chapter, we had shown that vinegar tempe had been found to be more pleasant and more acceptable among new consumers. Beside vinegar soaking, tempe had also been transformed into another food products such as tempe burger (Vital et al. 2018), seasoning powders (Gunawan-Puteri et al. 2015) and many more. However, these derivative products completely transformed the product and does not provide a solution for removing the unpleasant smell in the original tempe products.

One effect observed from the vinegar soaking process is the reduction of nutritional metabolites such as amino acids metabolites. Therefore, it is possible that another processing strategy can be adapted to alleviate the reduction of amino acid metabolites in vinegar soaked tempe. For this purpose, metabolomic analysis can be utilized to compare the effect of different processing strategies (Putri et al. 2022). Metabolomic analysis can provide rich information about metabolite profiles from each processing strategies for comparison. The metabolomic data can also be correlated with sensory evaluation for further discussion of metabolite changes in the different tempe types.

One new processing strategy that can be introduced on top of the vinegar soaking is crushing process. The crushing process is a possible solution to increasing the intensity of nutritional metabolites in vinegar soaked tempe. Soybeans in its crushed form had been claimed to be more suitable for fermentation by microorganisms (Li et al. 2013).

Combining the crushing process with vinegar soaking could lead to a more nutritional tempe that is appealing to new consumers. Since crushing process is a physical process, measurement of tempe texture and color will be beneficial for the discussion of tempe's physical characteristic changes and its sensory evaluation. The objective of this study is to define the effects of physical crushing and vinegar soaking to physical characteristics, metabolome and sensory profiling.

## **3.2 Materials and Methods**

### **3.2.1 Sample preparation**

The Rapprima brand (Aneka Fermentasi Industri, Bandung, Indonesia) of starter culture was used in this study. The soybeans used for making tempe were commercial Japanese soybeans of the Yukihome variety (grown in Ishikari, Japan). The water used in the tempe making process is mineral water (Suntory, Tokyo, Japan), and commercial vinegar (kokumotsu) was purchased from Mizkan Ltd. (Handa, Japan). Tempe was processed according to the Rumah Tempe Indonesia (RTI) method (Figure 3.1), in which tempe is soaked and boiled in vinegar (50 mL/L of mineral water). Crushed processing was done by putting the boiled soybeans into a mixer (TESCOM, Tokyo, Japan) and dry mix for 3 minutes. The tempe was packed, then fermented in an incubator (EYELA, Tokyo, Japan) at 30 °C for 48 h until the desired texture was achieved.

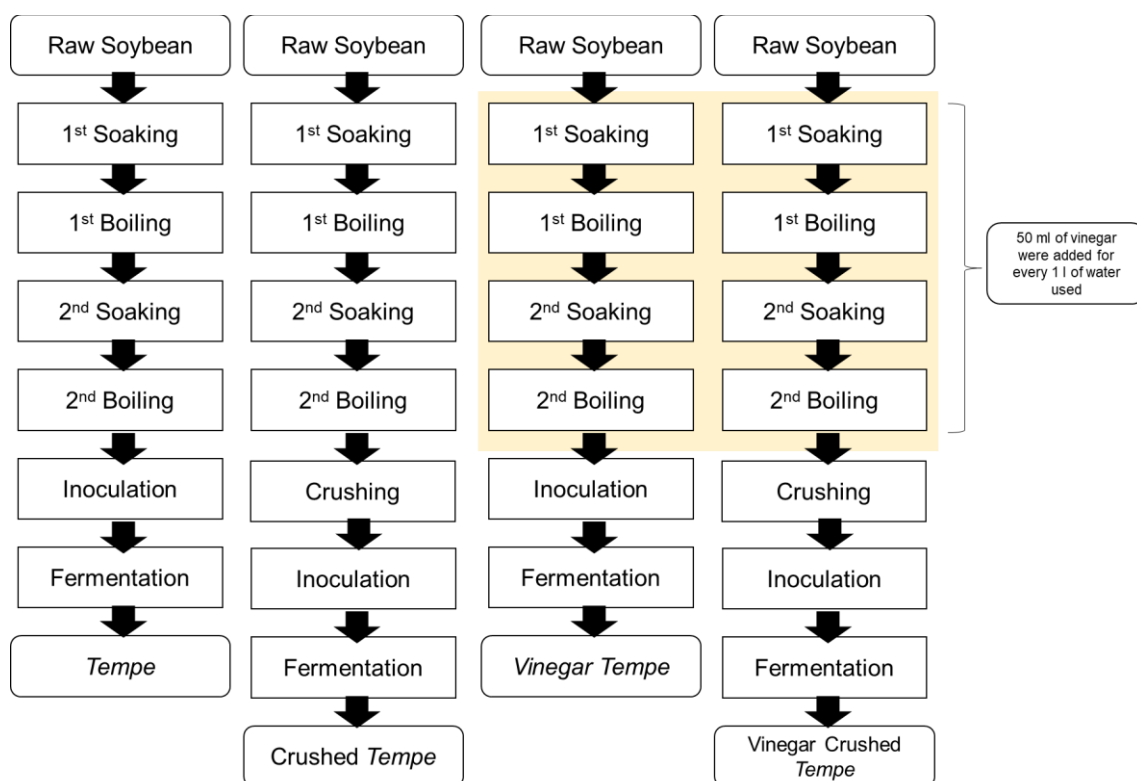


Figure 3.1 The tempe making process for control tempe, crushed tempe, vinegar tempe and vinegar crushed tempe.

### 3.2.2 Metabolite extraction and derivatization

The metabolite extraction and derivatization in this study is similar as reported in chapter 2. Fermented tempe were lyophilized and milled into a fine powder prior to extraction process. A 15 mg of lyophilized tempe was vortex-mixed with 1 mL of a 5:2:2 (v/v/v) ratio of methanol, ultrapure water (Wako Pure Chemical Industries, Osaka, Japan), chloroform (Kishida Chemical Co. Ltd., Osaka, Japan) and the internal standard ribitol (100 µg/mL).

The mixture was centrifuged at 4 °C and 11,740× *g* for 3 min, then 400 µL of supernatant was mixed with ultra-pure water (400 µL) in 1.5-mL microtubes and vortexed to induced separation between the polar and non-polar phases. The mixture was then centrifuged for

3 min at 4 °C, 11,740× *g* for 3 min. A total of 400 µL of aqueous phase was transferred to fresh 1.5 mL microtubes with holed caps. A total of 200 µL of the aqueous phase from all samples were mixed to make the Pooled quality controls (QCs). The solvent was evaporated with a Spin Dryer Standard (Taitec Co., Kyoto, Japan) for 2 h at room temperature. The evaporated samples were then lyophilized overnight (Taitec Co.). The samples were derivatized prior to GC/MS analysis. A total of 100 µL Methoxyamine hydrochloride (20 mg/mL in pyridine) was added to the lyophilized samples and incubated at 30 °C and 161× *g* for 90 min. After incubation, 50 µL N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) (GL Sciences Inc., Tokyo, Japan) was added and the mixture was incubated at 37 ° C and 161× *g* for 30 min before GC-MS analysis.

### **3.2.3 GC/MS analysis**

Metabolites of the tempe were analyzed using GC/MS-QP2010 (Shimadzu, Kyoto, Japan) equipped with a 0.25 × 30 mm, 0.25-µm Inert Cap 5 MS/NP column (GL Sciences Inc., Tokyo, Japan), and an AOC-20i/s autosampler (Shimadzu, Kyoto, Japan). The GC/MS conditions were based on a previous study of coffee metabolomics (Jumhawan et al. 2013). The reference for retention time is based on a standard alkane mixture (C8–C40) that was injected at the beginning of the sample analysis.

### **3.2.4 Processing of metabolomic data**

The GC/MS data were converted into ANDI files (\*.cdf) using GCMS Solution software (Shimadzu, Kyoto, Japan). The converted files were then analyzed using MS-DIAL v. 3.70 (Riken, Kanagawa, Japan) where peaks were detected, baseline was corrected, and peak retention times were aligned (Tsugawa et al. 2015). The peaks were annotated based on the retention index and MS information found in the GL-Science Database. The GL-Science Database is a public metabolite library shared by RIKEN. The relative intensity of each metabolite was normalized by comparison with that of the internal standard as an explanatory variable. Data were analyzed using multivariate analysis via SIMCA-P+ v.13 (Umetrics, Umeå, Sweden). The metabolite class for each annotated metabolite was determined by referring to the Human Metabolome Database.

### **3.2.5 Measurement of moisture content, texture and color analysis**

Measurement of moisture content, texture analysis and color analysis in this study were done in 5 biological replicates. Moisture content in this study was performed by calculating the percentage difference between mass before and after 3 h of oven drying (Mauer and Bradley 2017). Color measurements were performed using a Konica Minolta Spectrophotometer CM-2500d. The color measurement was done using the LAB color system. The color analysis was usually performed to correlate with consumer perception (Wrolstad and Smith 2017)

Texture analysis was performed using a 50 mm diameter cylindrical press jig on the Shimadzu EZ Test EZ-SX system with Shimadzu Trapezium X software ver. 1.5.4. Texture parameter analyzed in this study are hardness, cohesiveness, springiness, and

adhesiveness. These texture parameters are important texture parameter for consumer perception (Nishinari and Fang 2018).

### **3.2.6 Rate-all-that-apply (RATA) test**

A panel of 26 assessors (average age, 27.4 y; female, n = 11; male, = 15; Japanese, n = 17; non-Japanese, n = 9). All panelist were asked whether they were familiar with tempe sensory characteristics during the selection. Regardless of tempe familiarity, all panelist participate in a training session lasting between 30 and 60 min to familiarize themselves with 5-basic taste and the remaining 12 sensory attributes of tempe. Tempe were cut into cubes, cooked in an air fryer (Innsky, Shenzhen, China) for 5 min at 200 °C and served in plastic cups labeled with random three-digit codes.

In RATA test, 4 types of tempe samples (Control, Crushed, Vinegar, and Vinegar Crushed; each sample having 3 cubes of cooked tempe for each panelist) along with a test questionnaire with list of 17 sensory attributes. The arrangement of the 4 tempe samples were randomized for each panelist. Attributes rating were done on a 5-point intensity scale. The test questionnaire is shown in Figure S6. The list of the 17 sensory attributes were based on previous chapter with a modification by adding an additional “aftertaste” attribute (Table S6). The data of RATA test were treated as intensity as suggested (Meyners, Jaeger, and Ares 2016) and were found to be non-normal distribution. The data was analysed with Kruskal-Wallis with Dunn test for statistical comparison and visualized with boxplot. A web-based application (BoxPlotR) was used to make the boxplots.

### **3.2.7 Correlation between tempe metabolites and RATA sensory attributes**

Biplot and Variable Influence Projection (VIP) metabolites were obtained via Orthogonal Projection to Latent Structures (OPLS). Biplot in OPLS was performed by correlating significant sensory attributes (Y-variables) with metabolite data (X-variables). VIP metabolites for each significant sensory attributes were obtained by correlating each significant sensory attributes (Y-variable) with metabolites. The best OPLS model in this study were obtained the VIP was obtained by bootstrapping method (Alexander, Tropsha, and Winkler 2015). The best bootstrapping strategy in this study was performed by removing the vinegar tempe from the bootstrap samples. OPLS in this study was performed in SIMCA-P+ version 13 (Umetrics, Umea, Sweden).

## **3.3 Results and Discussion**

### **3.3.1 Metabolite profile of control, vinegar, crushed and crushed vinegar tempe**

All four types of tempe; control, vinegar soaked, crushed and vinegar crushed tempe finish their fermentation in 48 h. Previous chapter shows that vinegar soaking delayed tempe fermentation. However, the tempe batches in this study regardless of vinegar soaking or not was able to display the desired texture in 48 h fermentation. One possible reason for this is possibly due to soybean variation. Although this study is using the same brand of commercial soybeans used in previous chapter, it is possible that the batch of soybean had different phenotypes. Other possible variables were controlled in this study.

All 4 types of tempe samples were analyzed by GC/MS. A total of 199 metabolites were annotated, including 90 unknowns (Table S5). The processed GC/MS dataset was

assessed using multivariate analysis, and the difference between tempe samples were determined by principal component analysis (PCA) and heatmap (Figure 3.2).

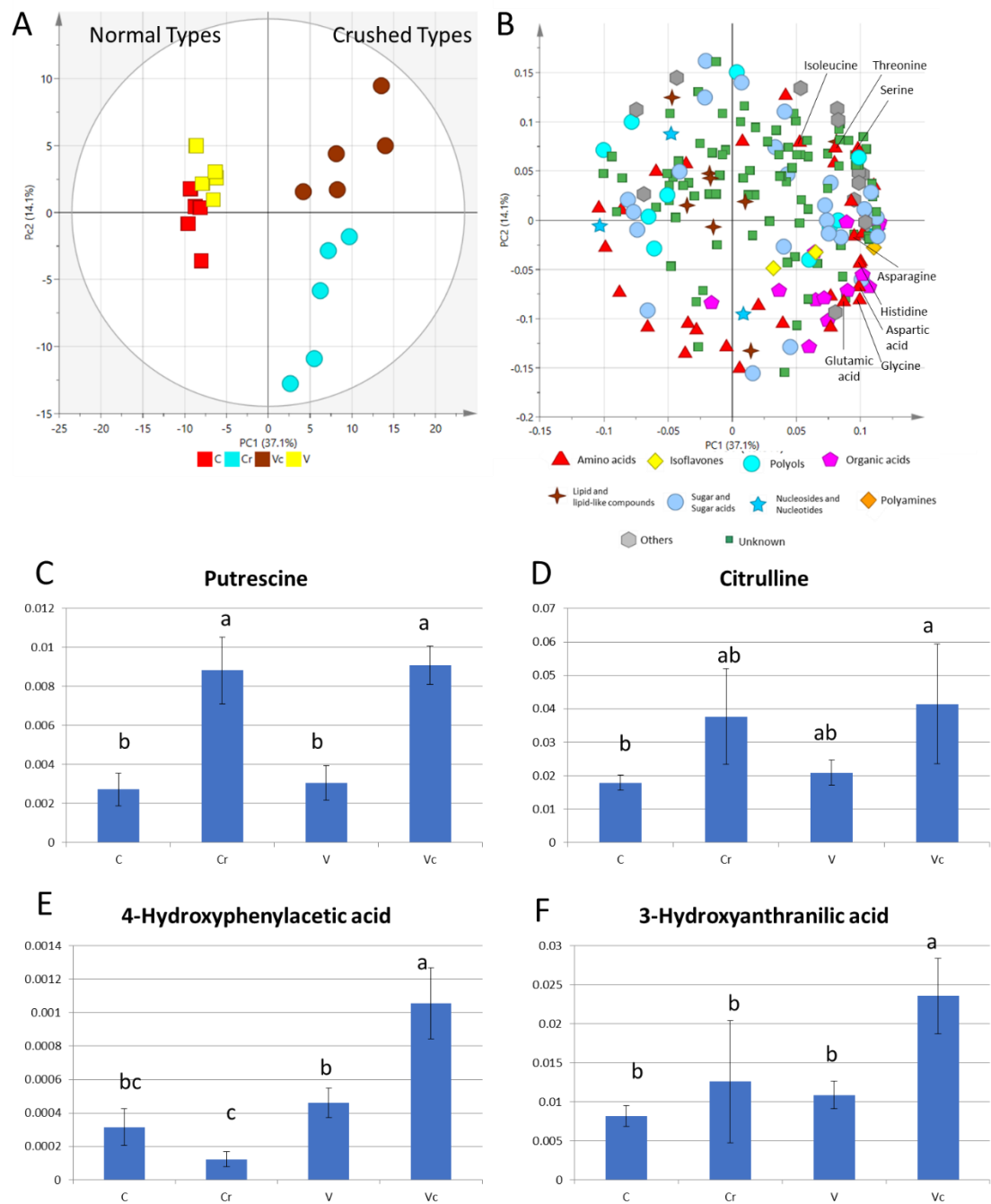


Figure 3.2 – Principal component analysis and bar graph of selected metabolites from the GC/MS analysis all tempe types. (A) score plot and (B) loading plot of all tempe

metabolites. Bar graph of (C) putrescine, (D) citrulline, (E) 4-hydroxyphenylacetic acid and (F) 3-hydroxyanthranilic acid. Different letters above bar chart denotes significant different ( $P < 0.05$ ) between samples in Tukey test. The sample term “C” denotes control tempe, “Cr” denotes crush tempe, “V” denotes vinegar tempe and “Vc” denotes vinegar crush tempe.

The results of the PCA shows a variation of 37.1 % and 14.1 % for PC1 and PC2, respectively (Figure 3.2A). Based on the group position of the samples, the axis of PC1 separates between two groups: group of control and vinegar tempe and group of crushed and crushed vinegar tempe. The group of control and vinegar tempe can be termed as normal type tempe samples and the group of crushed and crushed vinegar tempe can be termed as crushed type tempe samples. The axis of PC2 shows the differences between soaking the tempe with and without (control) vinegar for both groups of tempe.

Figure 3.2B shows the PCA loading plots. Majority of the metabolites, including those of amino acids, isoflavones, sugar and sugar acids, polyamines, and organic acids, were positioned on the positive side of PC1. Meanwhile, majority of polyols and nucleosides were positioned on the negative side of PC1. The class of the metabolites detected coincided with list of metabolites in previous chapter. The loading value of all metabolites is available in Table S7.

Among the metabolites that are increased due to crushing process are amino acid class metabolites. As seen in loading plot (Figure 3.2B), isoleucine, threonine, serine, asparagine, histidine, aspartic acid, glycine and glutamic acid are among the amino acids that are higher in both crushed tempe and vinegar crushed tempe. Besides amino acids,

the metabolite putrescine is also higher in both crushed and vinegar crushed tempe (Figure 3.2C). Putrescine is a polyamine that has antioxidant and anti-inflammatory capabilities (Munoz-Esparza et al. 2019). It has been suggested that dietary polyamine can contribute toward reducing the risk of cardiovascular disease (Soda 2010).

Another metabolite, citrulline had been found to be highest in vinegar crushed tempe followed by crushed tempe (Figure 3.2D). Citrulline is a beneficial metabolite that had potential to be part of therapy for inflammatory bowel disease (Ho et al. 2022). Another metabolites that had been detected in tempe is 4-hydroxyphenylacetic acid. Similar to putrescine, 4-hydroxyphenylacetic acid is a food metabolite that also has antioxidant properties (Sari et al. 2019). The crushing process seems to reduce the intensity of 4-hydroxyphenylacetic acid as seen in crushed tempe. However, combination of vinegar soaking and crushing process lead to significantly increase of 4-hydroxyphenylacetic acid (Figure 3.2E).

Combination of vinegar soaking and crushing process had also led to high intensity of 3-hydroxyanthranilic acid as seen crushed vinegar tempe (Figure 3.2F). Presence of this antioxidant metabolite in tempe had been previously reported (Esaki et al. 1996). However, this study report for the first time the processing strategy to increase this metabolite.

From this result, we can infer that crushing the soybeans can lead to increased intensity of primary metabolites in the fermented tempe. However, combination of both crushing and vinegar soaking can also lead to increment of specific beneficial metabolites in tempe. Therefore, the strategy to introduce crushing process or combination of crushing and vinegar soaking process can lead to a more nutritional tempe.

### 3.3.2 Texture analysis, moisture content and color analysis of control, vinegar, crushed and crushed vinegar tempe

The previous section described the effect of vinegar, crushing and combination of the two processes to the metabolite profile of tempe. In this section, we compared the physical characteristics of the tempe samples. The result of texture analysis, moisture content and color analysis is shown in Figure 3.3.

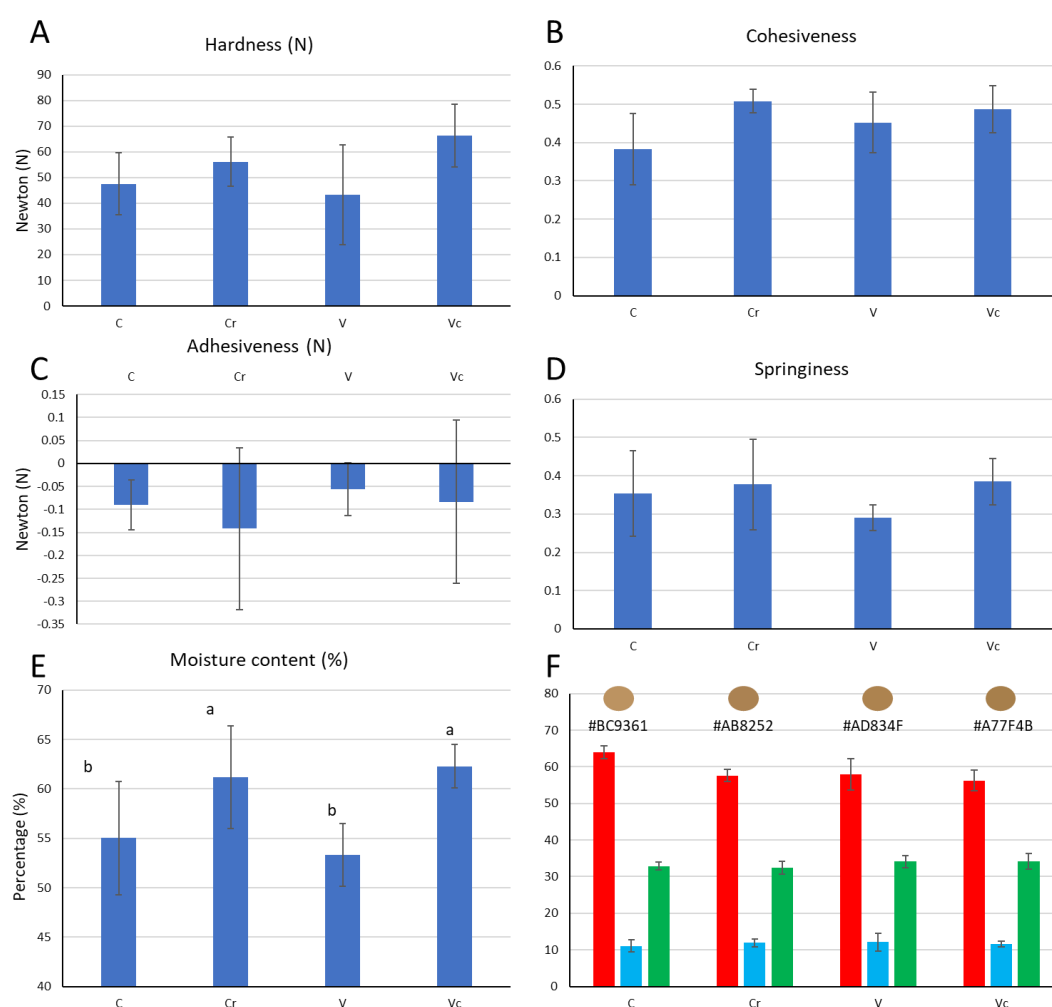


Figure 3.3 – Physical characteristics of control tempe, crushed tempe, vinegar tempe and vinegar crushed tempe. Bar graph (A-D) are textural properties, (E) moisture content and (F) color analysis of all four tempe types. The sample term “C” denotes control tempe,

“Cr” denotes crush tempe, “V” denotes vinegar tempe and “Vc” denotes vinegar crush tempe.

Figure 3A-3D shows the texture analysis parameter: hardness, cohesiveness, adhesiveness, and springiness. The results shows that the crushing process influence the texture process. This can be seen from the hardness, cohesiveness, and adhesiveness of the Crushed type of tempe sample (crushed tempe and vinegar crushed tempe) is higher than normal type tempe samples (control and vinegar tempe). The vinegar soaking process seems to influence the springiness of the tempe texture. This can be seen both vinegar and vinegar crushed tempe had lower springiness than both control and crushed tempe. However, there was no statistically significant difference ( $p > 0.05$ ) for all textural attributes among the four types of tempe.

Figure 3.3E shows the moisture content of the studied tempe. All four tempe were within the moisture content range of *tempe* (Ahnan-Winarno et al. 2021). The crushing process enabled more moisture to be accumulated within tempe. Both Crushed and Vinegar Crushed tempe had higher moisture content than control and vinegar tempe ( $p < 0.05$ ). The high moisture content of tempe samples is the reason for high texture variability since previous study had suggested that food with liquid content will have high texture variability (Bui and Hibbert 2014).

Figure 3.3F shows the measurement of cooked tempe color intensity. Color analysis is one of the important physical quality attributes of food and can be correlated with sensory analysis (Wrolstad and Smith 2017). The color measurement is based on the  $L^*a^*b^*$  color system.  $L^*$  indicates lightness;  $a^*$  and  $b^*$  are the chromaticity coordinates ( $+a^*$  indicates red direction,  $-a^*$  indicates green direction,  $+b^*$  indicates yellow direction, and

-b\* indicates blue direction). The value for each L\*a\*b\* can be converted to HEX color codes which is shown in above part of the Figure 3.3F. Based on the result, Control tempe has lighter brown color compared to other tempe types. This is possibility due to higher L\* value of Control tempe than the other tempe types. The tempe color intensity is within the range of the color coordinates reported by other studies on tempe (Handoyo and Morita 2006).

Based on these results, physical crushing influences the hardness, cohesiveness, adhesiveness, and springiness of the tempe. The physical crushing also significantly influenced the moisture content of the crushed type tempe sample. Vinegar soaking only influence the springiness of the tempe where both vinegar soaked tempe were less springy than normal type tempe samples. As for color analysis, each modified processing lead to darker brown color of tempe when compared to control tempe.

### **3.3.3 RATA sensory of tempe samples**

We investigated the sensory difference by performing Rate-All-That-Apply (RATA) test on control, crushed, vinegar and vinegar crushed tempe. The objective of the RATA test is to determine the intensity of selected sensory attributes (Meyners, Jaeger, and Ares 2016). Table S6 shows the 17 sensory attributes included in the RATA test. These attributes are based on the categories of color (golden-brown), texture (compact and crispy), aroma (beany, tempe-specific, alcohol, pleasant, yeast, and ammonia), taste (sweet, sour, salty, bitter, umami, astringent, aftertaste), and hedonic (acceptance).

Among the 26 panelist which consists of 17 Japanese and 9 South east Asian, only 3 Japanese panelist answered not familiar with tempe sensory characteristics during the

panelist recruitment stage. RATA test has been suggested to be best performed with large number of untrained panelist (Ares et al. 2014). However, another study had shown that RATA test with reduced number of panelists that had undergo training of sensory attributes is also credible and reproducible (Giacalone and Hedelund 2016). Therefore, all panelist in this study undergoes sensory training prior to actual RATA test to familiarize themselves with tempe and its sensory characteristics.

Table 3.1 shows the characteristics of the 26 panelists and the descriptive statistics of the sensory attributes in the test. Based on the descriptive statistics (Table 3.1) all sensory attributes were non-normally distributed ( $p < 0.05$ , Shapiro-Wilk tests). This is possibly due to different preference of sensory attributes by panelist from different country. In this case, panelist from different countries will have different sensory preference on tempe's sensory attributes.

Table 3.1 Characteristics and descriptive statistics of all RATA panelists

| Sensory Attributes | Tempe Types | Mean  | Std. Deviation | P-value of Shapiro-Wilk | P-value of Kruskal-Wallis Test |
|--------------------|-------------|-------|----------------|-------------------------|--------------------------------|
| Golden-brown       | Control     | 2.846 | 0.784          | 0.002                   | < .001                         |
|                    | Crushed     | 3.615 | 1.023          | 0.003                   |                                |
|                    | Vinegar     | 3.577 | 0.987          | 0.007                   |                                |
|                    | Vinegar     | 3.923 | 0.891          | 0.002                   |                                |
|                    | Crushed     |       |                |                         |                                |
| Compact            | Control     | 3.385 | 0.941          | 0.007                   | 0.472                          |
|                    | Crushed     | 3.615 | 1.235          | 0.001                   |                                |
|                    | Vinegar     | 3.346 | 0.936          | 0.001                   |                                |
|                    | Vinegar     | 3.654 | 0.846          | 0.001                   |                                |
|                    | Crushed     |       |                |                         |                                |
| Crispy             | Control     | 2.231 | 0.71           | < .001                  | 0.767                          |
|                    | Crushed     | 2.077 | 0.977          | < .001                  |                                |
|                    | Vinegar     | 2.231 | 1.142          | 0.004                   |                                |
|                    | Vinegar     | 2.308 | 1.087          | 0.009                   |                                |
|                    | Crushed     |       |                |                         |                                |
| Beany              | Control     | 3.308 | 0.97           | 0.018                   | 0.299                          |

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|                                |         |       |       |        |       |
|--------------------------------|---------|-------|-------|--------|-------|
| (Continued from previous page) |         |       |       |        |       |
|                                | Crushed | 3.192 | 0.981 | 0.034  |       |
|                                | Vinegar | 3.615 | 0.852 | 0.002  |       |
|                                | Vinegar | 3.154 | 1.084 | < .001 |       |
|                                | Crushed |       |       |        |       |
| Specific tempe                 | Control | 3.192 | 0.849 | < .001 | 0.321 |
|                                | Crushed | 3.154 | 0.834 | < .001 |       |
|                                | Vinegar | 3.154 | 1.008 | 0.001  |       |
|                                | Vinegar | 3.577 | 1.065 | 0.001  |       |
|                                | Crushed |       |       |        |       |
| Alcohol                        | Control | 1.538 | 0.706 | < .001 | 0.359 |
|                                | Crushed | 1.962 | 0.999 | < .001 |       |
|                                | Vinegar | 1.577 | 0.809 | < .001 |       |
|                                | Vinegar | 1.808 | 1.021 | < .001 |       |
|                                | Crushed |       |       |        |       |
| Pleasant                       | Control | 3.192 | 1.059 | 0.002  | 0.036 |
|                                | Crushed | 2.538 | 0.905 | 0.003  |       |
|                                | Vinegar | 3.154 | 1.12  | 0.045  |       |
|                                | Vinegar | 2.692 | 1.011 | 0.011  |       |
|                                | Crushed |       |       |        |       |
| Yeast                          | Control | 2.5   | 0.812 | 0.003  | 0.79  |
|                                | Crushed | 2.615 | 0.804 | 0.003  |       |
|                                | Vinegar | 2.538 | 0.905 | 0.007  |       |
|                                | Vinegar | 2.654 | 0.892 | < .001 |       |
|                                | Crushed |       |       |        |       |
| Ammonia                        | Control | 1.346 | 0.562 | < .001 | 0.027 |
|                                | Crushed | 1.846 | 1.19  | < .001 |       |
|                                | Vinegar | 1.577 | 0.809 | < .001 |       |
|                                | Vinegar | 2.115 | 1.071 | 0.001  |       |
|                                | Crushed |       |       |        |       |
| Sweet                          | Control | 1.923 | 0.688 | < .001 | 0.087 |
|                                | Crushed | 1.923 | 0.891 | < .001 |       |
|                                | Vinegar | 2.269 | 0.827 | 0.002  |       |
|                                | Vinegar | 1.692 | 0.788 | < .001 |       |
|                                | Crushed |       |       |        |       |
| Sour                           | Control | 2.231 | 1.142 | 0.004  | 0.078 |
|                                | Crushed | 2.269 | 1.218 | 0.003  |       |
|                                | Vinegar | 2.010 | 1.2   | < .001 |       |
|                                | Vinegar | 2.808 | 1.201 | 0.044  |       |
|                                | Crushed |       |       |        |       |
| Salty                          | Control | 2.462 | 0.905 | 0.003  | 0.937 |
|                                | Crushed | 2.462 | 0.948 | 0.004  |       |
|                                | Vinegar | 2.462 | 1.067 | 0.005  |       |
|                                | Vinegar | 2.654 | 1.231 | 0.025  |       |
|                                | Crushed |       |       |        |       |
| Bitter                         | Control | 2.038 | 1.038 | < .001 | 0.825 |

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|               |                    |       |       |        |       |
|---------------|--------------------|-------|-------|--------|-------|
|               | Crushed            | 1.962 | 0.871 | 0.001  |       |
|               | Vinegar            | 1.885 | 0.909 | < .001 |       |
|               | Vinegar<br>Crushed | 2.154 | 1.047 | 0.002  |       |
| Umami         | Control            | 2.885 | 0.993 | 0.038  | 0.706 |
|               | Crushed            | 2.923 | 0.935 | 0.02   |       |
|               | Vinegar            | 2.654 | 0.892 | < .001 |       |
|               | Vinegar<br>Crushed | 2.731 | 1.079 | < .001 |       |
| Astringent    | Control            | 2.269 | 1.002 | 0.003  | 0.799 |
|               | Crushed            | 2.346 | 1.129 | 0.004  |       |
|               | Vinegar            | 2.115 | 1.107 | < .001 |       |
|               | Vinegar<br>Crushed | 2.385 | 1.203 | 0.005  |       |
| Aftertaste    | Control            | 2.885 | 0.766 | 0.001  | 0.001 |
|               | Crushed            | 3.577 | 0.809 | < .001 |       |
|               | Vinegar            | 2.654 | 0.936 | 0.002  |       |
|               | Vinegar<br>Crushed | 3.462 | 0.989 | 0.001  |       |
| Acceptability | Control            | 3.038 | 0.894 | 0.018  | 0.003 |
|               | Crushed            | 2.615 | 0.898 | 0.003  |       |
|               | Vinegar            | 3.346 | 0.797 | 0.002  |       |
|               | Vinegar<br>Crushed | 2.538 | 0.811 | < .001 |       |

The non-normal distribution RATA results were then undergo Kruskal-Wallis test with Dunn's test for Multiple Comparison test (Dinno 2015). The non-parametric multiple comparison test shows that there are 5 sensory attributes that are statistically significant: golden-brown color, pleasant smell, ammonia smell, aftertaste, and acceptability (Table 3.1). Figure 3.4 shows the significant sensory attributes plotted in boxplots for detailed data visualization to avoid misleading during discussion of the average sensory score (Sidel, Stone, and Bloomquist 1981). The boxplot of non-significant sensory attributes are shown in Figure S7.

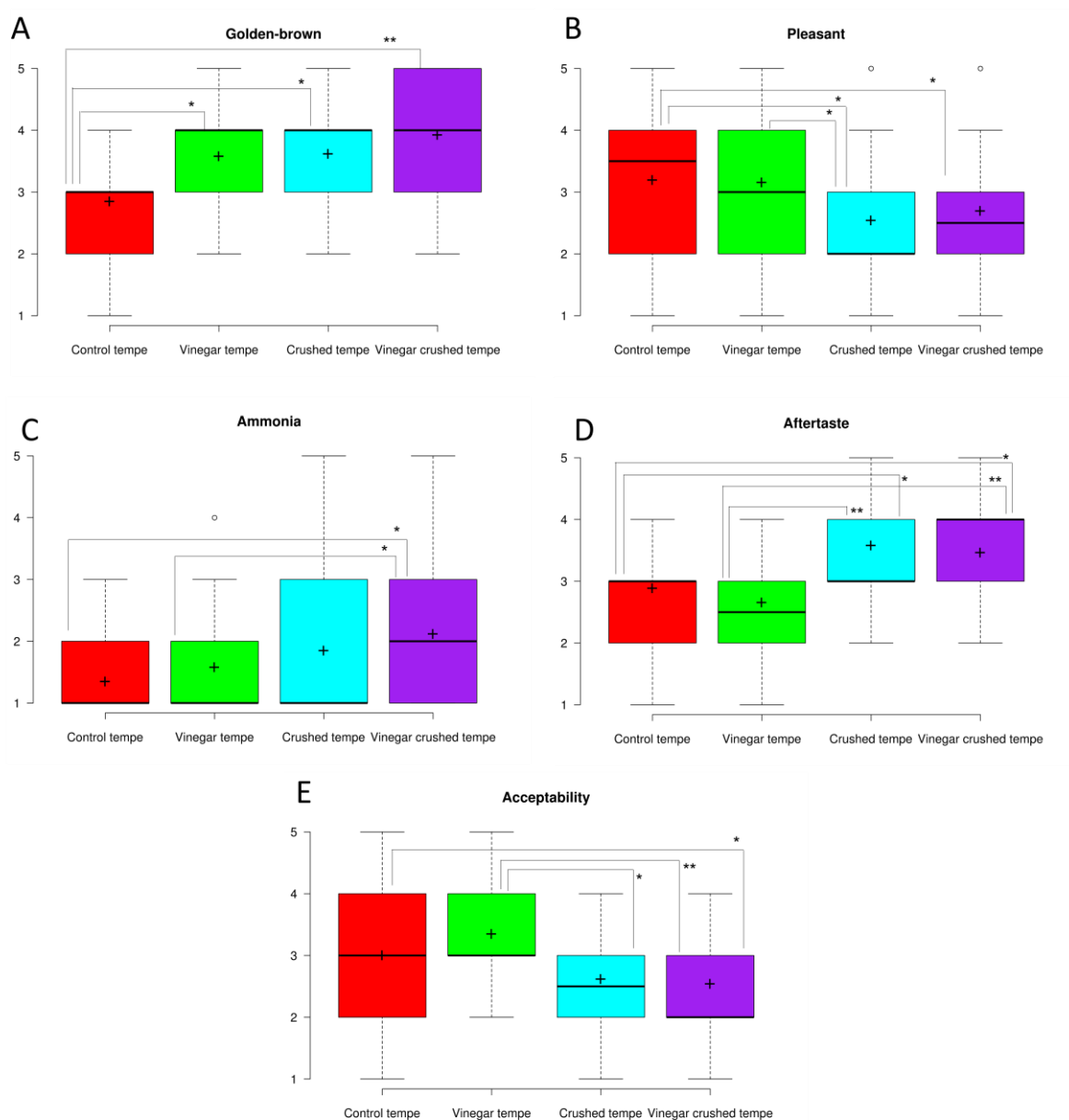


Figure 3.4 – Boxplots of significant RATA sensory attributes. The symbol “\*” denotes significant different in Kruskal Wallis with Dunn test. (“\*” denotes  $p < 0.05$  while “\*\*” denotes  $p < 0.01$ .)

The panelist felt that vinegar crushed tempe had the highest golden-brown color intensity (Figure 3.4A). Meanwhile, control tempe has the lowest golden-brown color intensity felt by the panelists. This indicate that control tempe has the lightest golden-brown color.

Statistically, control tempe is significantly different than vinegar and vinegar crushed tempe ( $p < 0.01$ ). These results are similar to the color analysis where color of control tempe is different from all other tempe types (Figure 3.3F).

Panelist felt that control tempe has the highest intensity of pleasant smell followed by vinegar, vinegar crushed and crushed tempe (Figure 3.4B). Although previous chapter had shown that vinegar tempe had more pleasant smell, there were no significant different between the control tempe and vinegar tempe. The different result from previous study is possibly due to increase number of tempe samples and the apparent effect of crushing process toward tempe's pleasant smell. Crushed tempe types (crushed tempe and vinegar crushed tempe) had significantly lower pleasant intensity ( $p < 0.05$ ) than both normal tempe types. This shows that vinegar soaking can mask the unpleasant smell of tempe.

The crushing treatment led to increase of ammonia smell in tempe. The panelist felt that vinegar crushed had the highest ammonia smell followed by crushed tempe (Figure 3.4C). Vinegar crushed tempe is significantly different from both vinegar and control tempe ( $p < 0.05$ ). In contrast, panelist felt that vinegar and control tempe had lowered intensity of ammonia smell. The increased intensity of metabolites in crushed tempe types (figure 3.2B) is the reason for higher ammonia intensity felt by the panelists.

The aftertaste in this study means the lingering taste in the mouth even after the tempe had already been swallowed. Tempe with the highest aftertaste is crushed tempe followed by vinegar crushed tempe (Figure 3.4D). There were no significant different between control and vinegar tempe ( $p > 0.05$ ), but crushing process contribute to significant different between normal type and crushed type tempe samples ( $p < 0.05$ ). One possible

explanation about the high aftertaste intensity in both crushed and vinegar crushed tempe is due to high intensity of amino acid metabolites (Sugimoto et al. 2020).

The acceptability sensory trait evaluates the overall acceptance of the panelist to the tempe. The highest acceptable felt by our panelists is vinegar tempe followed by control tempe (Figure 3.4E). Although it was not statistically different but the results is similar in previous chapter. The vinegar tempe is significantly different to both crushed and vinegar crushed tempe ( $p < 0.05$ ). The lowest acceptable tempe is vinegar crushed tempe, followed by crushed tempe.

Based on the RATA test, panelist felt that crushed type tempe samples (crushed tempe and vinegar crushed tempe) had higher ammonia smell, darker golden-brown and higher aftertaste. Tempe with the highest acceptance is vinegar tempe followed by control tempe. These normal type tempe samples has higher pleasant smell and lower sensory traits exhibited by the crushed type tempe samples.

#### **3.3.4 Correlation between physical characteristics, RATA and metabolite profiles.**

The texture analysis of the tempe in Figure 3.3A - 3.3D showed no significant differences among all four tempe types ( $p > 0.05$ ). The panelist also felt no significant different ( $p > 0.05$ ) for texture related sensory attributes; compact and crispiness as shown in table 3.1. Based on these results, the crushing process performed on tempe does not seem to exert influence on both textural characteristics and sensory attributes. However, it is certain that the impact of crushing process is more toward the metabolites of tempe.

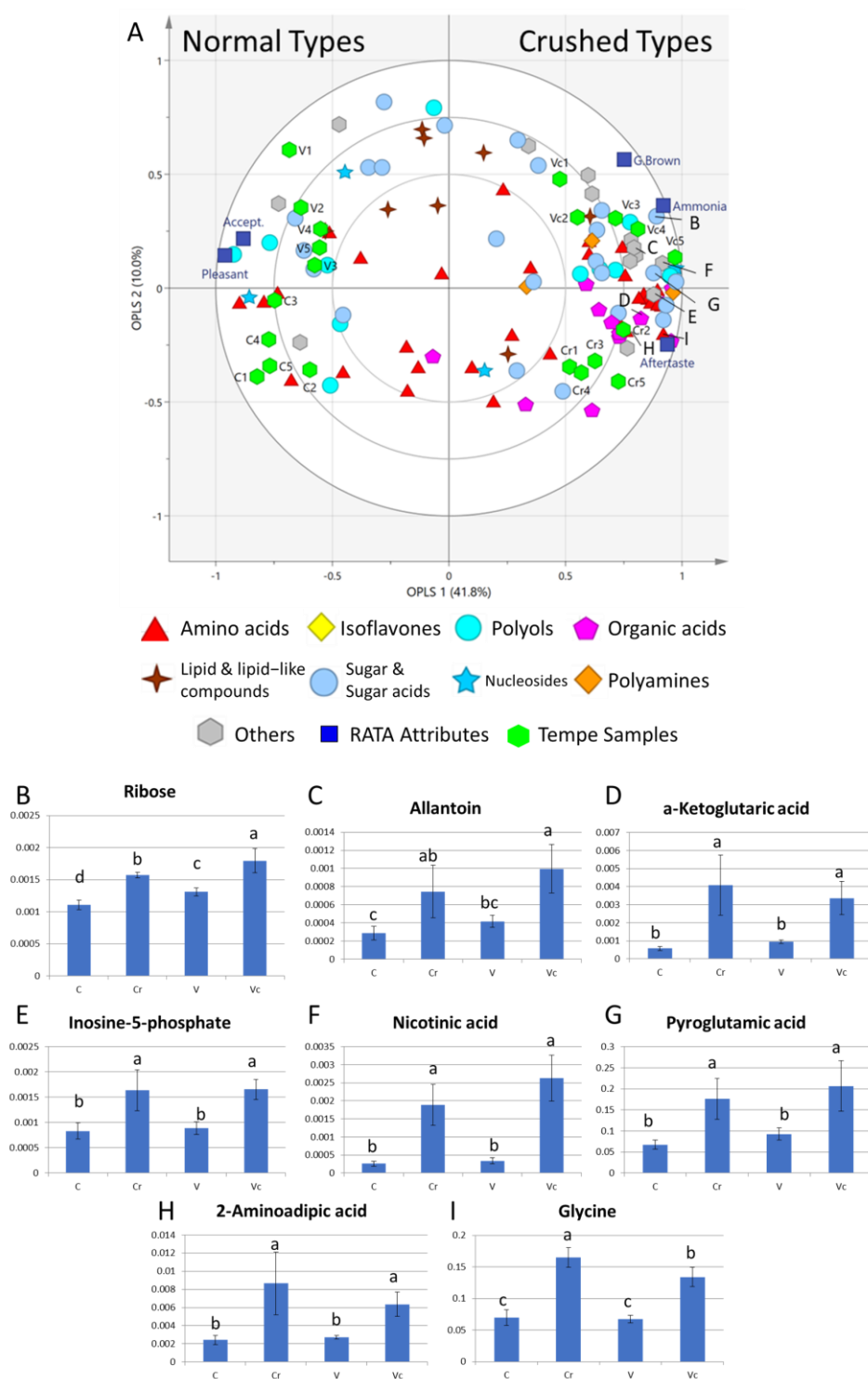


Figure 3.5 (A) Biplot of RATA attributes with tempe metabolites and bar graph shows sensory related VIP metabolites (B) ribose, (C) allantoin, (D)  $\alpha$ -ketoglutaric acid, (E) inosine-5-phosphate, (F) nicotinic acid, (G) pyroglutamic acid, (H) 2-aminoadipic acid

and (I) glycine. Different letters above bar chart denotes significant different ( $P < 0.05$ ) between samples in Tukey test. The sample term “C” denotes control tempe, “Cr” denotes crush tempe, “V” denotes vinegar tempe and “Vc” denotes vinegar crush tempe.

To investigate the correlation between tempe metabolites and RATA, OPLS analysis was performed by using metabolites intensity data and significant sensory attributes. The biplot analysis (Figure 3.5A) was performed to graphically project the variables and the observations as vectors and points respectively, onto a 2D principal component axes to simultaneously demonstrate the clustering of observations and correlations of the variables (Gabriel 1971). To further the discussion of metabolites, VIP metabolites were obtained from OPLS models (Figure S8). The table of VIP metabolites for each significant sensory attribute are shown in Table S8 – S12.

The biplot shows the golden-brown, ammonia and aftertaste sensory attributes are located on the positive side of the biplot. This coincides with the position of both crushed and vinegar crushed tempe (Figure 3.5A). The pleasant and acceptability sensory attributes are positioned on the negative side of the biplot. This position coincides with the position of control and vinegar tempe. Based on the position of individual metabolites, majority of the metabolites are positioned on the positive side of biplot. This shows that these metabolites is in higher intensity in crushed type tempe samples and contribute to the 3 sensory attributes (golden-brown, ammonia and aftertaste attributes). In contrast, the reduction of the metabolites in positive side of biplot seems to contribute toward the pleasant and acceptability sensory attribute.

Figure 3.5 B-I shows the selected VIP metabolites from OPLS model of significant sensory attributes. One of the VIP metabolites in golden-brown attribute is ribose (Figure

3.5B). Ribose had been found to contribute toward non-enzymatic browning with amino acids (Hemmler et al. 2018). Ribose is positioned in the positive side of the PC1 and PC2; the position of the darkest golden-brown tempe; vinegar crushed tempe. Interestingly, control tempe had the lowest ribose intensity which also correlates with color measurement, in which control tempe had the lightest brown intensity (Figure 3.3F). It is probable that the light golden-brown color in control tempe is due to reduce intensity of ribose as well as reduced amino acid metabolites as compared to the crushed type tempe samples.

Two metabolites; allantoin (Figure 3.5C) and  $\alpha$ -ketoglutaric acid (Figure 3.5D) are among the VIP metabolites for pleasant and ammonia smell attributes. Allantoin is a purine metabolite that can breakdown into ammonia (Ma et al. 2016, Fujiwara and Noguchi 1995). Crushing process increase the intensity of allantoin in crushed tempe. However, combination of crushing and vinegar soaking increase the intensity further (Figure 3.5C).  $\alpha$ -ketoglutaric acid had been found to contribute to the odour and flavour of dry fermented sausage (Herranz et al. 2004). The crushing process contributes toward increment of  $\alpha$ -ketoglutaric acid in both crushed tempe and crushed vinegar tempe (Figure 3.5D). From these results, we can deduce that although metabolites that can elicit pleasant smell is available in tempe, the pleasant effect could be masked by metabolites that exhibit ammonia smell.

There are 5 metabolites among the VIP metabolites that has relation to food sensory attributes in other published studies. Inosine-5-phosphate (Figure 3.5E) is a metabolite that can react synergistically with other amino acids to elicit umami taste (Kawai, Okiyama, and Ueda 2002). Nicotinic acid (Figure 3.5F) is a metabolite that had been

found to contribute to bitterness taste in coffee brew (Fujimoto et al. 2021). Pyroglutamic acid (Figure 3.5G) is a metabolite that had been identified to be correlated with taste in soy-sauce (Kong et al. 2018). The metabolite 2-aminoadipic (Figure 3.5H) had been correlated with the taste of chicken soup (Pérez-Palacios et al. 2017). These 4 metabolites are higher in both crushed tempe and crushed vinegar tempe. The fifth metabolite, glycine is one of the amino acid that can be emulate the taste of crabmeat (Fuke and Konosu 1991). Glycine is highest in crushed tempe followed by crushed vinegar tempe (Figure 3.5I). It seems that vinegar treatment reduced the content of glycine in crushed vinegar tempe. These 5 metabolites are assumed to contribute to the aftertaste attributes in tempe. Considering all the significant sensory attributes and the correlated metabolites, the increment of selected metabolites lead to darker golden-brown, higher ammonia smell and aftertaste in crushed tempe and crushed vinegar tempe led to reduce acceptance by the panelist. In contrast, lighter golden-brown, higher pleasant smell and reduced aftertaste is what made the control tempe and ultimately, vinegar tempe accepted by the panelist.

### **3.4 Conclusion**

In summary, introducing crushing process led to increase of primary metabolites in tempe. Combination of crushing process and vinegar soaking also lead to increase of specific nutritional metabolites such as citrulline, 4-hydroxyphenylacetic acid and 3-hydroxyanthranilic acid. The combination of crushing process and vinegar soaking process increases the tempe's nutritional value. Aside from metabolites, the crushing process also influence the textural properties, moisture content and color intensity of the

tempe. However, the increase of metabolites due to crushing process led to higher ammonia smell, reduced pleasant smell and strong aftertaste in tempe. These characteristics led to reduce acceptance of both crushed and vinegar crushed tempe by our sensory panelist. As for comparison between control and vinegar tempe, the vinegar tempe has higher acceptance by our sensory panelist. Based on these results, it is certain that crushing process can increase the nutritional value of tempe. However, this process reduced its acceptability even when combined with vinegar soaking process.

## **Chapter 4**

### **Conclusion**

The general objective of this research is to investigate processing strategies that can improve tempe sensory characteristics via a metabolomic approach. The first processing strategy evaluated in this study is the effect of vinegar soaking on its metabolome and sensory properties. The vinegar soaking process led to the reduction of primary metabolites especially amino acid metabolites in tempe. The reduction of these metabolites led our panelists to rate vinegar tempe as lighter golden-brown color, less ammonia smell, and less salty taste. The higher intensity and combination of several sensory-related metabolites such as 2-hydroxypyridine and N- $\alpha$ -acetyl ornithine led our sensory panelist to rate vinegar tempe higher in terms of pleasant smell and ultimately acceptability. Since the majority of our panelists are new consumers of tempe, we conclude that the vinegar soaking process is a feasible processing strategy for introducing tempe to new consumers with no prior knowledge of its sensory characteristics.

The second processing strategy evaluated is the combination of vinegar soaking with the soybean crushing process. One of the effects of vinegar soaking during tempe making is the decrease of nutritional metabolites such as amino acids. The introduction of the soybean crushing process to tempe increased its nutritional value. The combination of both processing also led to a new strategy to increase several specific metabolites such as citrulline, 4-hydroxyphenylacetic acid and 3-hydroxyanthranilic acid where these metabolites contribute beneficial nutritional value to tempe. However, the crushing process lowered the tempe sensory acceptance even after the combination with vinegar

soaking. This is because the increase of metabolites via the crushing process contributes to a higher ammonia smell and stronger aftertaste. Regarding this, both control and vinegar tempe which has lowered ammonia smell and lighter aftertaste have higher acceptance by our group of sensory panelists. Vinegar tempe has the highest acceptance followed by control tempe. Although unintentional, the result of the second study shows the replicability of the vinegar soaking process as a feasible processing strategy for introducing tempe to new consumers.

In a conclusion, the research found two processing strategies that can improve tempe in two separate qualities. The introduction of the vinegar soaking process can improve tempe's sensory characteristics while the introduction of the crushing process can improve tempe's nutritional quality. The combination of both processing strategies leads to a higher nutritional value of tempe. However, further food processing or more public health education for consumers is recommended to make the vinegar crushed tempe more acceptable to new consumers.

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## **List of Publication**

### **Original Research Paper:**

Hadi Akbar Dahlan, Y. Nambu, S. P. Putri, and E. Fukusaki, "Effects of Soaking Tempe in Vinegar on Metabolome and Sensory Profiles," *Metabolites*, vol. 12, p. 30, 2022.

### **In Preparation:**

Hadi Akbar Dahlan, S. P. Putri, and E. Fukusaki, "Effect of Physical crushing and Vinegar soaking to metabolome, physical characteristics and sensory profiles". (In preparation for submission to *Journal of Bioscience and Bioengineering*)

## Supplementary Figures and Tables

Control



24 h



48 h

Vinegar-treated



24 h



48 h



72 h

Figure S1. Progression of fungal growth during fermentation.

Control sample fermented for (A) 24 and (B) 48 h. Sample soaked in vinegar for 24 (C), 48 (D), and 72 (E) h.

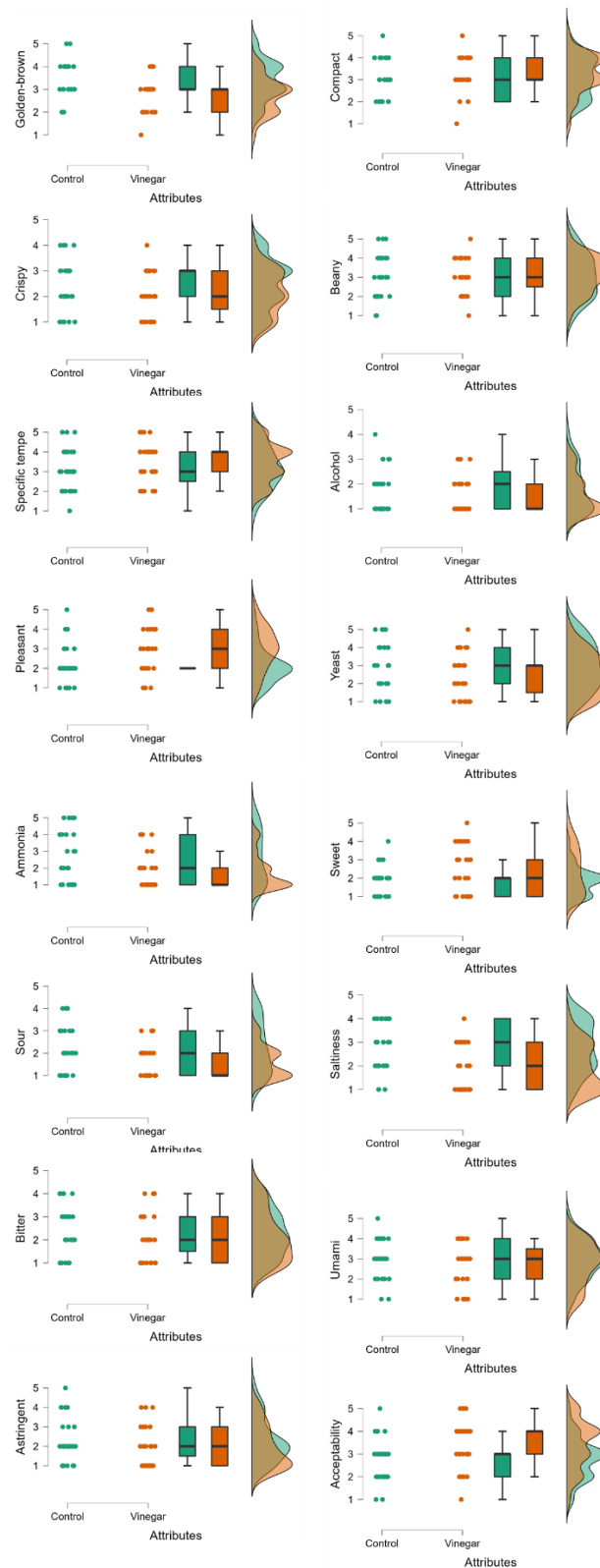


Figure S2. Raincloud plots of all 16 sensory attributes.



Figure S3. Bar graph of all annotated metabolites in tempe soaked without (control) and with vinegar. (Continued to next page)

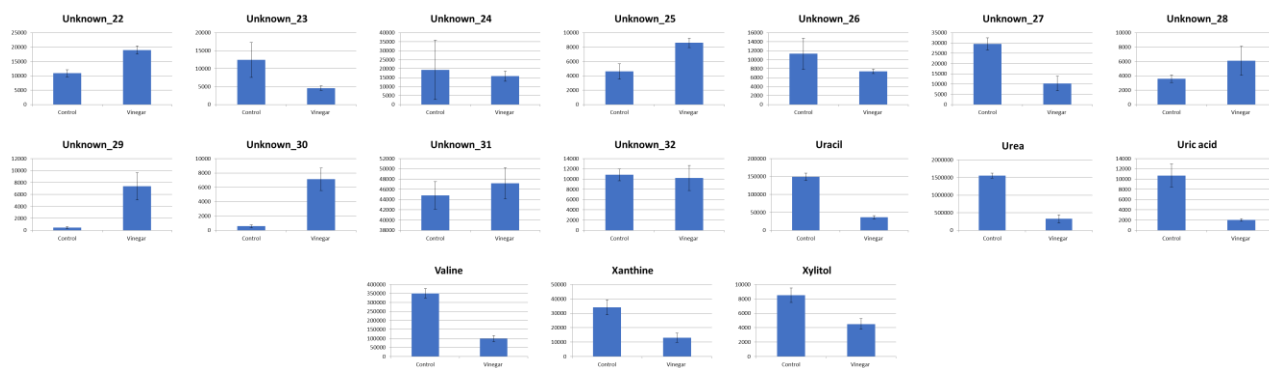


Figure S3. Bar graph of all annotated metabolites in tempe soaked without (control) and with vinegar. (Continued from previous page)

<Sensory evaluation Answer sheet>

Panel No. \_\_\_\_\_

Date (dd/mm/yy) \_\_\_\_\_

Sex    male   •   female \_\_\_\_\_

Age \_\_\_\_\_

NAME \_\_\_\_\_

Among the 3 tempe served, 2 out of 3 are the same, only 1 is different. Please choose the different one and circle the sample code. In addition, please describe what are the differences among the samples.

Sample set 1

| Sample No. |  |  |
|------------|--|--|
|            |  |  |

Please describe the differences.

\_\_\_\_\_

Sample set 2

| Sample No. |  |  |
|------------|--|--|
|            |  |  |

Please describe the differences.

\_\_\_\_\_

\* Thank you for your cooperation \*

Figure S4. Triangle test questionnaire

A

- Rate-All-That-Apply test
- Instruction**
- In front of you, there are 2 tempe samples. Please write the 3-digit code number in sample column. You can start to evaluate the sample from left to right. (テンペサンプルが2つあります。解答用紙に3桁のサンプル番号を記入して下さい。左から右の順にサンプルを評価して下さい)
  - Please carefully evaluate each sample by following this order (以下の順序に従って各サンプルを評価して下さい)
    - Observe the colour of the sample and mark the intensity based on your perception. (サンプルをよく観察して、感じた色について強度をチェックして下さい)
    - Sniff the sample for approximately 3 seconds. Three short sniffs are recommended (avoid long, deep inhalations). Keep your mouth closed while sniffing. Give interval around 30 seconds between sample. Sniff your own unscented arm as a blank between samples. (サンプルを約3秒間嗅いで下さい。注意：長く深く吸入しないで下さい。嗅ぐときは口を閉じて下さい。サンプル間のブランクとして自分の香りのない腕を嗅ぐなどして下さい)
    - Take one cube of sample, chew the sample with molar teeth for at least 5 times. let it in the mouth for 3 seconds before swallowing to feel the taste. (サンプルを一つとり、少なくとも5回噛んで下さい。飲み込む前に3秒間ほど味を感じて下さい)
    - Rinsing the palate (neutralizing) with water for each sample. Give interval between sample around 20-30 seconds. (サンプル間に水で口をリンスして下さい。サンプル間の間隔は20~30秒にして下さい)
  - Please finish the first sample before you start to evaluate the next one (次のサンプルに移る前に、最初のサンプルの評価を終了して下さい)
  - Please mark the intensity of attribute as (1) or very weak, if you fail to feel the attribute (感じなかった官能項目は強度を1: very weak にマークして下さい)
  - The score of attribute given for one sample is not relative to other sample. (官能項目の強度は他のサンプルとの相対値ではありません)
  - Please read the list of attributes carefully before you start to evaluate the sample (サンプルの評価を始める前に、官能項目のリストを注意深く読んで下さい)

B

Answer sheet

Name :  
Age/Gender :  
Date :

|                 | No | Attribute      | Very weak                | Weak                     | Medium                   | Strong                   | Very strong              |
|-----------------|----|----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                 |    |                | 1                        | 2                        | 3                        | 4                        | 5                        |
|                 |    |                |                          |                          |                          |                          |                          |
| Sample<br>code: | 1  | Golden-brown   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 2  | Compact        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 3  | Crispy         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 4  | Beany          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 5  | Specific tempe | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 6  | Alcohol        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 7  | Pleasant       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 8  | Yeast          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 9  | Ammonia        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 10 | Sweet          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 11 | Sour           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 12 | Salty          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 13 | Bitter         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 14 | Umami          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 15 | Astringent     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 16 | Acceptability  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

|                 | No | Attribute      | Very weak                | Weak                     | Medium                   | Strong                   | Very strong              |
|-----------------|----|----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                 |    |                | 1                        | 2                        | 3                        | 4                        | 5                        |
|                 |    |                |                          |                          |                          |                          |                          |
| Sample<br>code: | 1  | Golden-brown   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 2  | Compact        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 3  | Crispy         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 4  | Beany          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 5  | Specific tempe | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 6  | Alcohol        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 7  | Pleasant       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 8  | Yeast          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 9  | Ammonia        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 10 | Sweet          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 11 | Sour           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 12 | Salty          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 13 | Bitter         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 14 | Umami          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 15 | Astringent     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                 | 16 | Acceptability  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |

Figure S5. RATA test questionnaire in chapter 2.  
(A) Instructions for RATA test. (B) Answer sheet.

A

## Rate-All-That-Apply test

## Instruction

- In front of you, there are 4 tempe samples. Please write the 3-digit code number in sample column. You can start to evaluate the sample from left to right. (テンペサンプルが4つあります。解答用紙に3桁のサンプル番号を記入して下さい。左から右の順にサンプルを評価して下さい)
- Please carefully evaluate each sample by following this order (以下の順序に従って各サンプルを評価して下さい)
  - Observe the colour of the sample and mark the intensity based on your perception. (サンプルをよく観察して、感じた色について強度をチェックして下さい)
  - Sniff the sample for approximately 3 seconds. Three short sniffs are recommended (avoid long, deep inhalations). Keep your mouth closed while sniffing. Give interval around 30 seconds between sample. Sniff your own unscented arm as a blank between samples. (サンプルを約3秒間嗅いで下さい。注意：長く深く吸入しないで下さい。嗅ぐときは口を閉じていて下さい。サンプル間のブランクとして自分の香りのない腕を嗅ぐなどして下さい)
  - Take one cube of sample, chew the sample with molar teeth for at least 5 times. let it in the mouth for 3 seconds before swallowing to feel the taste. (サンプルを一つとり、少なくとも5回噛んで下さい。飲み込む前に3秒間ほど味を感じて下さい)
  - Rinsing the palate (neutralizing) with water for each sample. Give interval between sample around 20-30 seconds. (サンプル間に水で口をリンスして下さい。サンプル間隔は20～30秒にして下さい)
- Please finish the first sample before you start to evaluate the next one (次のサンプルに移る前に、最初のサンプルの評価を終了して下さい)
- Please mark the intensity of attribute as (1) or very weak, if you fail to feel the attribute (感じなかった官能項目は強度を1: very weak にマークして下さい)
- The score of attribute given for one sample is not relative to other sample. (官能項目の強度は他のサンプルとの相対値ではありません)
- Please read the list of attributes carefully before you start to evaluate the sample (サンプルの評価を始める前に、官能項目のリストを注意深く読んで下さい)

B

## Answer sheet

Name :  
Age/Gender :  
Date :

|                       | No | Attribute      | Very weak                | Weak                     | Medium                   | Strong                   | Very strong              |
|-----------------------|----|----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                       |    |                | 1                        | 2                        | 3                        | 4                        | 5                        |
| Sample code:<br>----- | 1  | Golden-brown   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 2  | Compact        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 3  | Crispy         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 4  | Beany          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 5  | Specific tempe | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 6  | Alcohol        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 7  | Pleasant       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 8  | Yeast          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 9  | Ammonia        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 10 | Sweet          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 11 | Sour           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 12 | Salty          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 13 | Bitter         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 14 | Umami          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 15 | Astringent     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 16 | Aftertaste     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 17 | Acceptability  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Comments              |    |                |                          |                          |                          |                          |                          |

|                       | No | Attribute      | Very weak                | Weak                     | Medium                   | Strong                   | Very strong              |
|-----------------------|----|----------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
|                       |    |                | 1                        | 2                        | 3                        | 4                        | 5                        |
| Sample code:<br>----- | 1  | Golden-brown   | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 2  | Compact        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 3  | Crispy         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 4  | Beany          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 5  | Specific tempe | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 6  | Alcohol        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 7  | Pleasant       | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 8  | Yeast          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 9  | Ammonia        | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 10 | Sweet          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 11 | Sour           | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 12 | Salty          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 13 | Bitter         | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 14 | Umami          | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 15 | Astringent     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 16 | Aftertaste     | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
|                       | 17 | Acceptability  | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> | <input type="checkbox"/> |
| Comments              |    |                |                          |                          |                          |                          |                          |

Figure S6. RATA test questionnaire in chapter 3.

(A) Instructions for RATA test. (B) Answer sheet

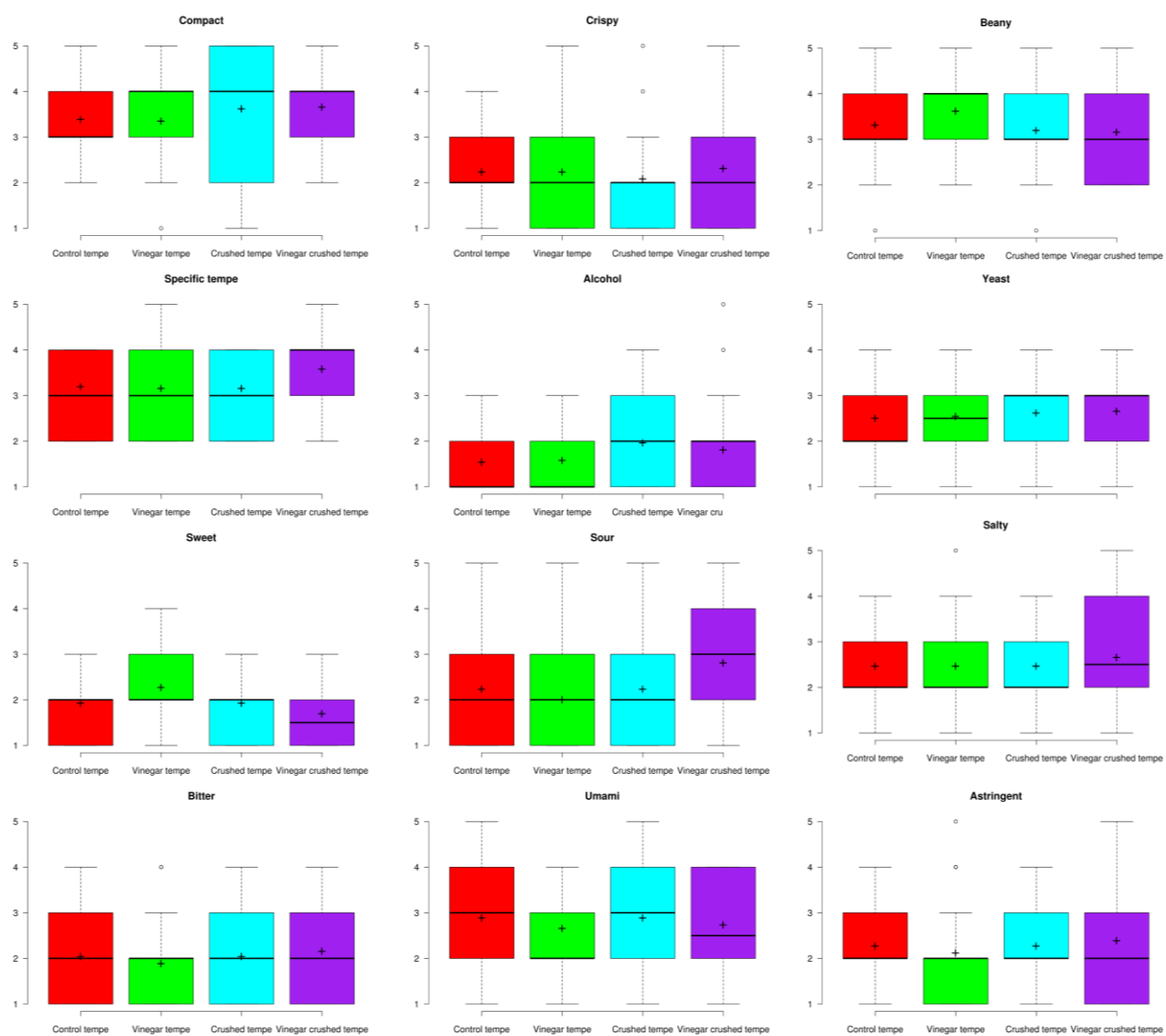


Figure S7. Boxplot of the non-significant Sensory attributes in Chapter 3

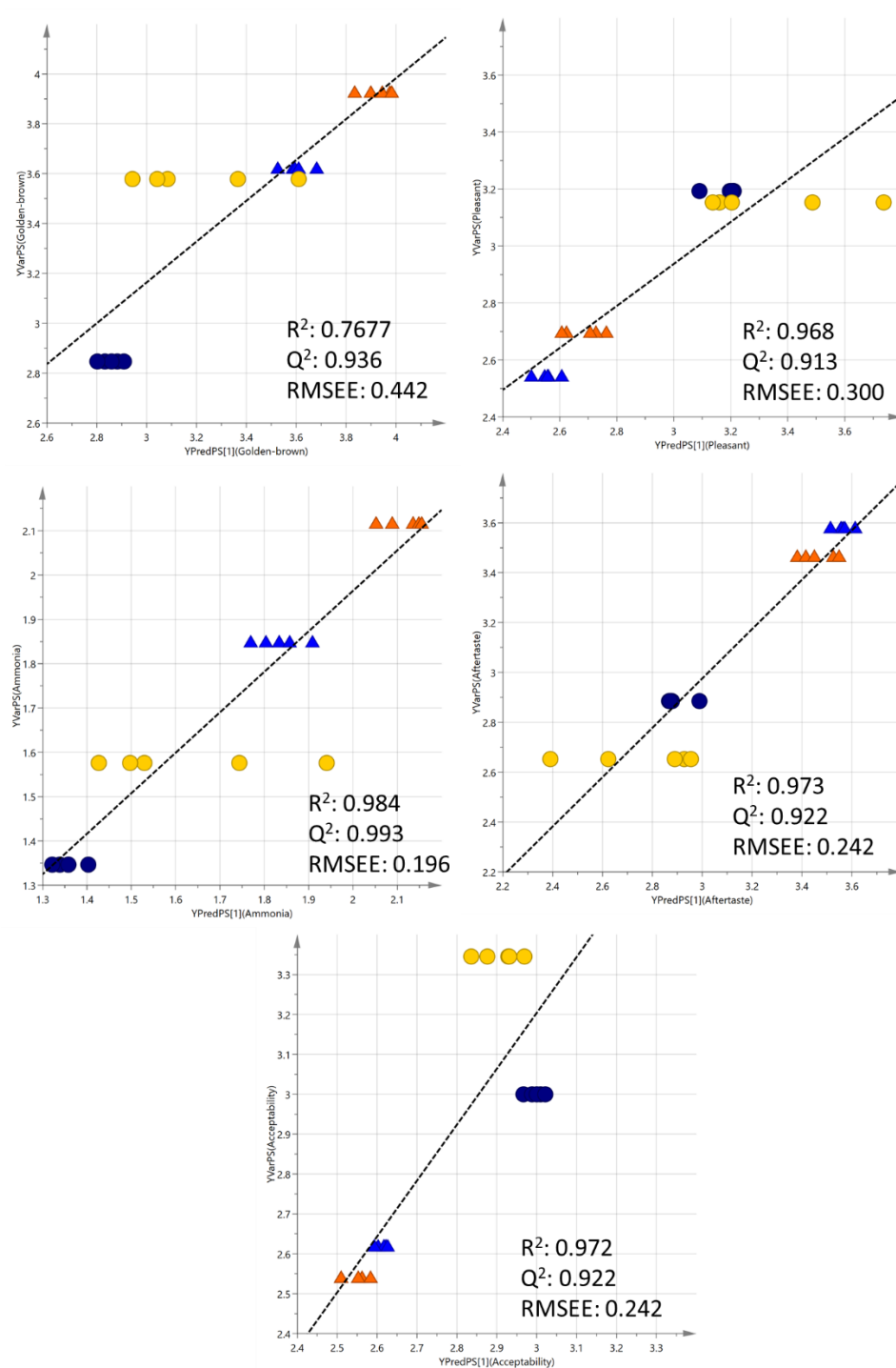


Figure S8. OPLS model of significant RATA sensory attributes in chapter 3

Table S1. Annotated peaks in all *tempe* samples in chapter 2 by GC/MS analysis

| No. | Compound name            | Average<br>RT (min) | Average<br>RI | Quant<br>mass | MSI<br>level |
|-----|--------------------------|---------------------|---------------|---------------|--------------|
| 1   | Unknown_1                | 4.244               | 987.52        | 221           | 4            |
| 2   | Unknown_2                | 4.597               | 1023.29       | 207           | 4            |
| 3   | n-Propylamine            | 4.647               | 1027.72       | 174           | 1            |
| 4   | Unknown_3                | 4.76                | 1037.71       | 117           | 4            |
| 5   | 2-Hydroxypyridine        | 4.776               | 1039.12       | 152           | 1            |
| 6   | Unknown_4                | 4.858               | 1046.41       | 117           | 4            |
| 7   | Oxalacetic acid+Pyruvate | 4.914               | 1051.33       | 174           | 1            |
| 8   | Lactic acid              | 5.062               | 1064.4        | 147           | 1            |
| 9   | Alanine                  | 5.545               | 1107.37       | 116           | 1            |
| 10  | Oxalate                  | 5.845               | 1134.93       | 147           | 1            |
| 11  | Unknown_5                | 5.856               | 1135.99       | 130           | 4            |
| 12  | Unknown_6                | 5.967               | 1146.22       | 147           | 4            |
| 13  | Unknown_7                | 6.311               | 1177.78       | 144           | 4            |
| 14  | 2-Aminobutyric acid      | 6.318               | 1178.47       | 130           | 1            |
| 15  | Unknown_8                | 6.363               | 1182.61       | 281           | 4            |
| 16  | Malonic acid             | 6.634               | 1208.07       | 147           | 1            |
| 17  | Unknown_9                | 6.767               | 1221.18       | 130           | 4            |
| 18  | Valine                   | 6.791               | 1223.58       | 144           | 1            |
| 19  | Urea                     | 6.939               | 1238.22       | 147           | 1            |
| 20  | Unknown_10               | 7.064               | 1250.54       | 147           | 4            |
| 21  | Unknown_11               | 7.087               | 1252.81       | 96            | 4            |
| 22  | Unknown_12               | 7.218               | 1265.7        | 110           | 4            |

|    |                     |       |         |     |   |
|----|---------------------|-------|---------|-----|---|
| 23 | 2-Aminoethanol      | 7.319 | 1275.77 | 174 | 1 |
| 24 | Leucine             | 7.358 | 1279.59 | 158 | 1 |
| 25 | Phosphate           | 7.385 | 1282.21 | 299 | 1 |
| 26 | Unknown_13          | 7.503 | 1293.87 | 129 | 4 |
| 27 | Isoleucine          | 7.581 | 1301.69 | 158 | 1 |
| 28 | Proline             | 7.624 | 1306.27 | 142 | 1 |
| 29 | Glycine             | 7.721 | 1316.63 | 174 | 1 |
| 30 | Glyceric acid       | 7.943 | 1340.3  | 147 | 1 |
| 31 | Uracil              | 8.003 | 1346.67 | 241 | 1 |
| 32 | Fumaric acid        | 8.019 | 1348.37 | 245 | 1 |
| 33 | Serine              | 8.223 | 1370.17 | 204 | 1 |
| 34 | Threonine           | 8.483 | 1397.9  | 218 | 1 |
| 35 | Unknown_14          | 8.519 | 1401.84 | 116 | 4 |
| 36 | Thymine             | 8.595 | 1410.52 | 255 | 1 |
| 37 | Unknown_15          | 8.746 | 1427.84 | 144 | 4 |
| 38 | b-Alanine           | 8.826 | 1437.02 | 174 | 1 |
| 39 | Malic acid          | 9.355 | 1497.6  | 147 | 1 |
| 40 | Unknown_16          | 9.451 | 1509.15 | 115 | 4 |
| 41 | Meso erythritol     | 9.571 | 1523.69 | 147 | 1 |
| 42 | Methionine          | 9.625 | 1530.36 | 176 | 1 |
| 43 | Aspartic acid       | 9.63  | 1530.89 | 232 | 1 |
| 44 | Pyroglutamic acid   | 9.657 | 1534.27 | 156 | 1 |
| 45 | 4-Aminobutyric acid | 9.719 | 1541.73 | 174 | 1 |
| 46 | Unknown_17          | 9.778 | 1548.92 | 155 | 4 |
| 47 | Pyrogallol          | 9.878 | 1561.19 | 147 | 1 |

|    |                             |        |         |     |   |
|----|-----------------------------|--------|---------|-----|---|
| 48 | Unknown_18                  | 9.938  | 1568.45 | 220 | 4 |
| 49 | 3-Hydroxy-3-methylglutarate | 10.301 | 1613.44 | 147 | 1 |
| 50 | Glutamic acid               | 10.423 | 1629.29 | 246 | 1 |
| 51 | Phenylalanine               | 10.524 | 1642.31 | 218 | 1 |
| 52 | Asparagine                  | 10.843 | 1683.52 | 116 | 1 |
| 53 | Arabionose                  | 10.854 | 1685.03 | 103 | 1 |
| 54 | Unknown_19                  | 10.876 | 1687.83 | 290 | 4 |
| 55 | Ribose                      | 10.972 | 1700.33 | 103 | 1 |
| 56 | Unknown_20                  | 11.018 | 1706.59 | 173 | 4 |
| 57 | 2-Aminoadipic acid          | 11.163 | 1726.33 | 260 | 1 |
| 58 | Xylitol                     | 11.216 | 1733.59 | 217 | 1 |
| 59 | Arabitol                    | 11.305 | 1745.63 | 217 | 1 |
| 60 | Putrescine                  | 11.39  | 1757.36 | 174 | 1 |
| 61 | Unknown_21                  | 11.44  | 1764.12 | 116 | 4 |
| 62 | Glutamine                   | 11.599 | 1785.77 | 156 | 1 |
| 63 | O-Phosphoethanolamine       | 11.726 | 1803.35 | 299 | 1 |
| 64 | Ornithine                   | 11.955 | 1836.24 | 142 | 2 |
| 65 | Isocitric acid+Citric acid  | 11.978 | 1839.48 | 273 | 1 |
| 66 | Homogentisate               | 12.045 | 1849.14 | 384 | 1 |
| 67 | Myristic acid               | 12.053 | 1850.29 | 117 | 1 |
| 68 | Unknown_22                  | 12.064 | 1851.81 | 147 | 4 |
| 69 | Pinitol                     | 12.161 | 1865.78 | 147 | 2 |
| 70 | Unknown_23                  | 12.171 | 1867.3  | 174 | 4 |
| 71 | Adenine                     | 12.297 | 1885.28 | 264 | 1 |

|    |                               |        |         |     |   |
|----|-------------------------------|--------|---------|-----|---|
| 72 | 3-Hydroxyanthranilic acid     | 12.303 | 1886.27 | 354 | 1 |
| 73 | Psicose+Tagatose              | 12.425 | 1903.85 | 103 | 1 |
| 74 | Sorbose                       | 12.431 | 1904.88 | 218 | 1 |
| 75 | Fructose                      | 12.491 | 1913.79 | 103 | 1 |
| 76 | Mannose                       | 12.526 | 1919.19 | 319 | 1 |
| 77 | Glucono-1,5-lactone           | 12.563 | 1924.65 | 217 | 1 |
| 78 | Galactose                     | 12.573 | 1926.14 | 319 | 1 |
| 79 | Glucose                       | 12.613 | 1932.23 | 319 | 1 |
| 80 | Lysine                        | 12.66  | 1939.37 | 174 | 1 |
| 81 | Histidine                     | 12.67  | 1940.8  | 154 | 1 |
| 82 | Galactose+Glucose             | 12.749 | 1952.69 | 319 | 1 |
| 83 | Tyrosine                      | 12.783 | 1957.88 | 218 | 1 |
| 84 | Sorbitol                      | 12.897 | 1974.99 | 319 | 1 |
| 85 | Unknown_24                    | 13.097 | 2005.42 | 217 | 4 |
| 86 | N- $\alpha$ -Acetyl ornithine | 13.167 | 2016.51 | 174 | 1 |
| 87 | Unknown_25                    | 13.19  | 2020.07 | 227 | 4 |
| 88 | Xanthine                      | 13.314 | 2039.68 | 353 | 1 |
| 89 | Gluconic acid                 | 13.334 | 2042.87 | 147 | 1 |
| 90 | Plamitic acid(16:0)           | 13.359 | 2046.85 | 117 | 1 |
| 91 | Glucarate                     | 13.434 | 2058.77 | 204 | 1 |
| 92 | N-Acetyl glucosamine          | 13.804 | 2117.87 | 319 | 1 |
| 93 | Uric acid                     | 13.854 | 2126.24 | 441 | 1 |
| 94 | Inositol                      | 13.889 | 2131.98 | 217 | 1 |
| 95 | Unknown_26                    | 14.192 | 2181.85 | 326 | 4 |
| 96 | Stearic acid(17:0)            | 14.561 | 2244.57 | 117 | 1 |

|     |                      |        |         |     |   |
|-----|----------------------|--------|---------|-----|---|
| 97  | Tryptophan           | 14.591 | 2249.69 | 202 | 1 |
| 98  | Unknown_27           | 15.171 | 2351.74 | 204 | 4 |
| 99  | Unknown_28           | 15.256 | 2366.95 | 217 | 4 |
| 100 | Fructose 6-phosphate | 15.278 | 2370.83 | 387 | 1 |
| 101 | Saccharopine         | 15.837 | 2474.04 | 357 | 1 |
| 102 | Unknown_29           | 16.021 | 2508.67 | 318 | 4 |
| 103 | Unknown_30           | 16.024 | 2509.22 | 315 | 4 |
| 104 | Adenosine            | 16.835 | 2669.39 | 230 | 1 |
| 105 | Sucrose              | 17.008 | 2704.42 | 361 | 1 |
| 106 | b-Lactose            | 17.37  | 2779.81 | 204 | 1 |
| 107 | Trehalose            | 17.545 | 2816.75 | 361 | 1 |
| 108 | Maltose              | 17.55  | 2817.89 | 160 | 1 |
| 109 | Lactitol             | 17.643 | 2837.77 | 204 | 1 |
| 110 | Melibiose            | 18.125 | 2943.04 | 204 | 1 |
| 111 | Daidzein             | 18.264 | 2973.88 | 398 | 1 |
| 112 | Genistein            | 18.33  | 2988.6  | 471 | 1 |
| 113 | Unknown_31           | 18.425 | 3010.18 | 119 | 4 |
| 114 | Unknown_32           | 18.525 | 3033.1  | 204 | 4 |
| 115 | Raffinose            | 20.947 | 3500.52 | 361 | 1 |

Table S2 Loading values of all detected and annotated metabolites in all *tempe* samples  
in chapter 2

| Compound name               | PC1        | PC2        |
|-----------------------------|------------|------------|
| 2-Aminoadipic acid          | 0.111251   | 0.0842888  |
| 2-Aminobutyric acid         | 0.119153   | 0.00612189 |
| 2-Aminoethanol              | 0.107882   | 0.0979546  |
| 2-Hydroxypyridine           | 0.0948885  | -0.125071  |
| 3-Hydroxy-3-methylglutarate | 0.115629   | 0.0436792  |
| 3-Hydroxyanthranilic acid   | 0.1171     | -0.046526  |
| 4-Aminobutyric acid         | 0.111383   | 0.0870558  |
| Adenine                     | 0.117442   | 0.0509399  |
| Adenosine                   | 0.117989   | 0.0368745  |
| Alanine                     | 0.113868   | 0.071394   |
| Arabionose                  | 0.09129    | 0.0885949  |
| Arabitol                    | -0.0167307 | -0.0185507 |
| Asparagine                  | 0.119397   | 0.0127304  |
| Aspartic acid               | 0.107106   | 0.0904442  |
| b-Alanine                   | 0.117453   | 0.0299369  |
| b-Lactose                   | 0.0400397  | 0.0514968  |
| Daidzein                    | 0.0387413  | -0.144559  |
| Fructose 6-phosphate        | 0.0982565  | -0.0956011 |
| Fructose                    | -0.0940741 | 0.0808738  |
| Fumaric acid                | 0.113477   | 0.0755294  |
| Galactose                   | 0.0174371  | 0.205638   |
| Galactose+Glucose           | 0.103485   | 0.0213879  |

|                            |            |             |
|----------------------------|------------|-------------|
| Genistein                  | 0.0381734  | -0.160969   |
| Glucarate                  | 0.102268   | -0.00137672 |
| Gluconic acid              | -0.0800893 | 0.0520314   |
| Glucono-1,5-lactone        | -0.0526163 | 0.0814631   |
| Glucose                    | 0.101459   | 0.0184966   |
| Glutamic acid              | 0.107649   | 0.0905721   |
| Glutamine                  | 0.100546   | -0.118649   |
| Glyceric acid              | 0.116701   | 0.0529339   |
| Glycine                    | 0.115669   | 0.0632151   |
| Histidine                  | 0.118904   | 0.0122085   |
| Homogentisate              | 0.106673   | 0.100847    |
| Inositol                   | 0.0398856  | 0.0623467   |
| Isocitric acid+Citric acid | 0.110387   | 0.0896823   |
| Isoleucine                 | 0.108974   | 0.0922514   |
| Lactic acid                | 0.046845   | 0.055777    |
| Lactitol                   | 0.0782785  | -0.0513179  |
| Leucine                    | 0.103685   | 0.104178    |
| Lysine                     | 0.120266   | 0.00800413  |
| Malic acid                 | 0.115704   | 0.059501    |
| Malonic acid               | -0.0684568 | 0.156124    |
| Maltose                    | 0.109726   | -0.0414208  |
| Mannose                    | 0.113786   | 0.0118298   |
| Melibiose                  | 0.102605   | 0.0926235   |
| Meso erythritol            | 0.108361   | -0.0848659  |
| Methionine                 | 0.106292   | 0.100169    |

|                               |            |             |
|-------------------------------|------------|-------------|
| Myristic acid                 | 0.0746097  | -0.17338    |
| N- $\alpha$ -Acetyl ornithine | 0.0323723  | -0.221236   |
| N-Acetyl glucosamine          | 0.110205   | 0.0898054   |
| n-Propylamine                 | -0.023146  | -0.0288336  |
| O-Phosphoethanolamine         | 0.119612   | -0.00845888 |
| Ornithine                     | 0.0565792  | -0.202731   |
| Oxalacetic acid+Pyruvate      | 0.107215   | -0.0498708  |
| Oxalate                       | 0.0488651  | -0.161012   |
| Phenylalanine                 | 0.103364   | 0.103206    |
| Phosphate                     | 0.0950168  | -0.140355   |
| Pinitol                       | 0.0888486  | -0.00364856 |
| Plamitic acid(16:0)           | 0.0584958  | -0.0115653  |
| Proline                       | 0.113466   | 0.0633126   |
| Psicose+Tagatose              | -0.0931397 | 0.0863125   |
| Putrescine                    | 0.114644   | 0.0421549   |
| Pyrogallol                    | 0.117954   | -0.0303011  |
| Pyroglutamic acid             | 0.106597   | -0.102292   |
| Raffinose                     | 0.0912791  | -0.0828948  |
| Ribose                        | 0.0920108  | -0.0266172  |
| Saccharopine                  | 0.115095   | 0.00760532  |
| Serine                        | 0.119054   | 0.00660174  |
| Sorbitol                      | 0.0573512  | 0.185008    |
| Sorbose                       | -0.0780573 | -0.0223337  |
| Stearic acid(17:0)            | 0.0738856  | -0.098529   |
| Sucrose                       | 0.0193285  | -0.135499   |

|            |             |            |
|------------|-------------|------------|
| Threonine  | 0.118996    | 0.0308335  |
| Thymine    | 0.114756    | -0.0636035 |
| Trehalose  | 0.114692    | -0.0574231 |
| Tryptophan | 0.10883     | 0.0932273  |
| Tyrosine   | 0.115487    | 0.0632281  |
| Unknown_1  | -0.0548093  | 0.101332   |
| Unknown_2  | -0.0491454  | 0.0957078  |
| Unknown_3  | 0.106348    | 0.0948749  |
| Unknown_4  | 0.103997    | 0.0880615  |
| Unknown_5  | -0.0626809  | 0.10256    |
| Unknown_6  | -0.00299234 | 0.0944071  |
| Unknown_7  | -0.0602622  | 0.104452   |
| Unknown_8  | 0.0958694   | -0.0365823 |
| Unknown_9  | -0.0566303  | 0.105248   |
| Unknown_10 | -0.0108009  | 0.0393845  |
| Unknown_11 | 0.00922927  | 0.0267223  |
| Unknown_12 | -0.0860988  | 0.0559862  |
| Unknown_13 | -0.0121771  | 0.0920822  |
| Unknown_14 | 0.102529    | 0.026246   |
| Unknown_15 | 0.0560653   | -0.0662544 |
| Unknown_16 | 0.118967    | 0.0224974  |
| Unknown_17 | 0.0819305   | -0.154704  |
| Unknown_18 | 0.112348    | 0.0789971  |
| Unknown_19 | 0.0289801   | 0.0417187  |
| Unknown_20 | 0.09086     | -0.144237  |

|            |           |            |
|------------|-----------|------------|
| Unknown_21 | 0.11603   | 0.0279105  |
| Unknown_22 | 0.0773565 | -0.175819  |
| Unknown_23 | 0.113374  | 0.0225347  |
| Unknown_24 | 0.0582328 | -0.0270243 |
| Unknown_25 | 0.0774671 | -0.170816  |
| Unknown_26 | 0.112837  | -0.0409979 |
| Unknown_27 | 0.1191    | 0.0250884  |
| Unknown_28 | 0.083352  | -0.159602  |
| Unknown_29 | 0.0303209 | -0.220389  |
| Unknown_30 | 0.0306054 | -0.217705  |
| Unknown_31 | 0.0387293 | -0.109984  |
| Unknown_32 | 0.0225348 | 0.0158149  |
| Uracil     | 0.115631  | 0.0661068  |
| Urea       | 0.113375  | 0.0755236  |
| Uric acid  | 0.113531  | 0.0635482  |
| Valine     | 0.111378  | 0.0769583  |
| Xanthine   | 0.118449  | 0.0261408  |
| Xylitol    | 0.119547  | 0.00417824 |

Table S3. List of 16 sensory attributes in Chapter 2

| Category      | No | Attribute             | Description                                                                                                            |
|---------------|----|-----------------------|------------------------------------------------------------------------------------------------------------------------|
| Color<br>色    | 1  | Golden-brown          | Typical <i>tempe</i> color ranging from light golden brown to dark golden brown (テンペの色。金色のような茶色の強さ)                    |
|               | 2  | Compact               | Compactness of bean (high score indicates extremely dense product such as fudge brownie) (豆のコンパクトさ。高いスコアは密度が高いことを意味する) |
| Texture<br>質感 | 3  | Crispy                | Easy to break into crumbs or pieces associated with potato crisps (ポテトチップスのように簡単に碎ける度合い)                               |
|               | 4  | Beany                 | Aroma associated with cooked legumes (調理された豆のような匂い)                                                                    |
| Aroma<br>香り   | 5  | Specific <i>tempe</i> | Intensity of “ <i>tempe</i> -specific” aroma (テンペ特有の匂い)                                                                |
|               | 6  | Alcohol               | Cold, pungent smell associated with alcohol and distilled spirits (アルコールや蒸留酒のような冷たく刺激のある匂い)                            |

|                       |    |           |                                                                                                               |
|-----------------------|----|-----------|---------------------------------------------------------------------------------------------------------------|
|                       | 7  | Pleasant  | Aroma with a good connotation<br>(いい匂い)                                                                       |
|                       | 8  | Yeast     | A sour, fermented aromatic commonly associated with yeast<br>(酵母のような酸味のある発酵の匂い)                               |
|                       | 9  | Ammonia   | Pungent described as sharp sensation of nasal mucous membranes associated with vinegar (お酢のような鼻の粘膜を刺激するような匂い) |
| <b>Taste</b><br><br>味 | 10 | Sweet     | The fundamental taste factor associated with a sucrose solution<br>(甘味。シヨ糖溶液のような味覚)                           |
|                       | 11 | Sour      | A fundamental taste factor of which citric acid in water is typical (酸味。クエン酸のような味覚)                           |
|                       | 12 | Saltiness | The fundamental taste factor associated with sodium chloride (salt) solution (塩味。塩化ナトリウムのような味覚)               |
|                       | 13 | Bitter    | A fundamental taste factor of which caffeine or quinine is                                                    |

|                      |    |               |                                                                                                                  |
|----------------------|----|---------------|------------------------------------------------------------------------------------------------------------------|
|                      |    |               | typical（苦味。カフェインまたはキニーネのような味覚）                                                                                   |
|                      | 14 | Umami         | The fundamental taste factor of monosodium glutamate is typical（旨味。グルタミン酸ナトリウムのような味覚）                            |
|                      | 15 | Astringent    | The drying, puckering sensation on the tongue and other mouth surfaces（舌の表面にしわが寄るようなちくちくする感覚）                    |
| <b>Hedonic</b><br>嗜好 | 16 | Acceptability | Term to describe acceptance of <i>tempe</i> as an overall value concurrent with the all attributes（テンペとしての総合的な値） |

Table S4 Profile of RATA panelist in chapter 2

| Panelist | Nationality | Familiarity |
|----------|-------------|-------------|
| P1       | JP          | NF          |
| P2       | JP          | NF          |
| P3       | JP          | NF          |
| P4       | JP          | NF          |
| P5       | JP          | NF          |
| P6       | JP          | NF          |
| P7       | JP          | NF          |
| P8       | JP          | NF          |
| P9       | JP          | NF          |
| P10      | JP          | NF          |
| P11      | JP          | NF          |
| P12      | JP          | NF          |
| P13      | JP          | NF          |
| P14      | JP          | NF          |
| P15      | JP          | NF          |
| P16      | JP          | NF          |
| P17      | ID          | F           |
| P18      | ID          | F           |
| P19      | ID          | F           |
| P20      | ID          | F           |
| P21      | ID          | F           |
| P22      | ID          | F           |
| P23      | TH          | F           |

|     |    |   |
|-----|----|---|
| P24 | JP | F |
| P25 | MY | F |
| P26 | ID | F |
| P27 | JP | F |

Familiarity means familiarity with *tempe* sensory characteristics. The letter “NF” represents Not familiar while the letter “F” represents familiar.

Table S5. Annotated peaks in all *tempe* samples in chapter 3 by GC/MS analysis

| No. | Compound name               | Average Rt | Average RI | Quant mass | MSI level |
|-----|-----------------------------|------------|------------|------------|-----------|
| 1   | Unknown                     | 4.184      | 967.23     | 134.0883   | 4         |
| 2   | Unknown                     | 4.203      | 969.8      | 117.0621   | 4         |
| 3   | Unknown                     | 4.27       | 979.06     | 147.1167   | 4         |
| 4   | Unknown                     | 4.33       | 987.26     | 221.0606   | 4         |
| 5   | Propyleneglycol             | 4.456      | 1002.97    | 117.0848   | 1         |
| 6   | Unknown                     | 4.844      | 1037.28    | 117.1      | 4         |
| 7   | 2-Hydroxypyridine           | 4.866      | 1039.22    | 152.0823   | 1         |
| 8   | Unknown                     | 4.925      | 1044.43    | 123.0259   | 4         |
| 9   | Unknown                     | 4.948      | 1046.42    | 117.1      | 4         |
| 10  | Oxalacetic acid+Pyruvate    | 5          | 1051.01    | 174.0547   | 1         |
| 11  | Unknown                     | 5.09       | 1058.99    | 147.1161   | 4         |
| 12  | Unknown                     | 5.136      | 1063.03    | 207.0094   | 4         |
| 13  | Lactic acid                 | 5.151      | 1064.33    | 147.1076   | 1         |
| 14  | Unknown                     | 5.391      | 1085.57    | 117.12     | 4         |
| 15  | Valine_1TMS_minor           | 5.433      | 1089.22    | 146.0857   | 1         |
| 16  | Alanine_2TMS                | 5.635      | 1107.33    | 116.1062   | 1         |
| 17  | Unknown                     | 5.807      | 1123.21    | 102.0887   | 4         |
| 18  | Unknown                     | 5.815      | 1123.94    | 133.1071   | 4         |
| 19  | Unknown                     | 6.057      | 1146.11    | 147.1046   | 4         |
| 20  | Leucine_1TMS                | 6.194      | 1158.69    | 86.13281   | 1         |
| 21  | 2-Aminobutyric acid         | 6.409      | 1178.48    | 130.1266   | 1         |
| 22  | Isoleucine_1TMS_minor       | 6.43       | 1180.47    | 86.12813   | 1         |
| 23  | Unknown                     | 6.454      | 1182.63    | 147.1083   | 4         |
| 24  | Unknown                     | 6.465      | 1183.65    | 241.0097   | 4         |
| 25  | Unknown                     | 6.665      | 1202.17    | 174.1121   | 4         |
| 26  | Unknown                     | 6.866      | 1222.04    | 117.0717   | 4         |
| 27  | Valine_2TMS                 | 6.883      | 1223.71    | 144.1703   | 1         |
| 28  | Urea                        | 7.017      | 1236.95    | 147.1077   | 1         |
| 29  | Unknown                     | 7.1        | 1245.18    | 166.0917   | 4         |
| 30  | Unknown                     | 7.181      | 1253.12    | 96.11364   | 4         |
| 31  | Serine_2TMS_minor           | 7.283      | 1263.26    | 116.0969   | 1         |
| 32  | Unknown                     | 7.353      | 1270.13    | 85.11739   | 4         |
| 33  | 2-Aminoethanol              | 7.413      | 1276.08    | 174.1125   | 1         |
| 34  | Leucine_2TMS                | 7.45       | 1279.78    | 158.1766   | 1         |
| 35  | Glycerol                    | 7.476      | 1282.27    | 147.1086   | 1         |
| 36  | Nicotinic acid              | 7.633      | 1297.85    | 180.0605   | 1         |
| 37  | Threonine_2TMS_minor        | 7.668      | 1301.41    | 117.1      | 1         |
| 38  | Isoleucine_2TMS_major       | 7.673      | 1301.94    | 158.1674   | 1         |
| 39  | Proline_2TMS                | 7.718      | 1306.74    | 142.1531   | 1         |
| 40  | Succinic acid(or anhydride) | 7.811      | 1316.65    | 147.1118   | 1         |
| 41  | Glycine_3TMS                | 7.816      | 1317.11    | 174.119    | 1         |

|    |                                |        |         |          |   |
|----|--------------------------------|--------|---------|----------|---|
| 42 | Glyceric acid                  | 8.034  | 1340.37 | 147.1196 | 1 |
| 43 | Uracil                         | 8.096  | 1347.01 | 241.0521 | 1 |
| 44 | Fumaric acid                   | 8.109  | 1348.36 | 245.0484 | 1 |
| 45 | Serine_3TMS_major              | 8.315  | 1370.3  | 204.1016 | 1 |
| 46 | Alanine_3TMS                   | 8.333  | 1372.24 | 188.1121 | 1 |
| 47 | 3-Pyridyl acetic acid          | 8.515  | 1391.54 | 117.1052 | 1 |
| 48 | Threonine_3TMS_major           | 8.576  | 1398.06 | 218.1047 | 1 |
| 49 | Glutaric acid(or anhydride)    | 8.649  | 1406.33 | 147.1214 | 1 |
| 50 | Thymine                        | 8.689  | 1410.82 | 255.0797 | 1 |
| 51 | Unknown                        | 8.85   | 1429.24 | 160.1093 | 4 |
| 52 | b-Alanine_3TMS                 | 8.921  | 1437.39 | 174.1145 | 1 |
| 53 | Unknown                        | 8.979  | 1443.95 | 174.1197 | 4 |
| 54 | Unknown                        | 8.989  | 1445.19 | 147.0945 | 4 |
| 55 | Unknown                        | 9.215  | 1470.92 | 174.1136 | 4 |
| 56 | Unknown                        | 9.292  | 1479.75 | 158.1306 | 4 |
| 57 | Unknown                        | 9.348  | 1486.15 | 232.0969 | 4 |
| 58 | Malic acid                     | 9.449  | 1497.74 | 147.1109 | 1 |
| 59 | Unknown                        | 9.477  | 1501.03 | 158.1    | 4 |
| 60 | Unknown                        | 9.533  | 1507.83 | 174.1197 | 4 |
| 61 | Threitol                       | 9.595  | 1515.36 | 103.0636 | 1 |
| 62 | Unknown                        | 9.65   | 1522.01 | 174.1258 | 4 |
| 63 | Meso erythritol                | 9.665  | 1523.82 | 147.1235 | 1 |
| 64 | Aspartic acid_3TMS             | 9.725  | 1531.13 | 232.1047 | 1 |
| 65 | Pyroglutamic acid_2TMS         | 9.755  | 1534.81 | 156.1016 | 1 |
| 66 | Unknown                        | 9.768  | 1536.35 | 174.1036 | 4 |
| 67 | Hydroxyproline                 | 9.793  | 1539.46 | 230.1167 | 1 |
| 68 | 4-Aminobutyric acid            | 9.816  | 1542.18 | 174.1172 | 1 |
| 69 | Unknown                        | 9.876  | 1549.46 | 155.1143 | 4 |
| 70 | Unknown                        | 9.932  | 1556.3  | 263.1767 | 4 |
| 71 | Unknown                        | 9.952  | 1558.71 | 120.1062 | 4 |
| 72 | Threonic acid                  | 9.975  | 1561.61 | 147.1173 | 1 |
| 73 | Unknown                        | 10.043 | 1569.88 | 243.1759 | 4 |
| 74 | $\alpha$ -ketoglutaric acid    | 10.156 | 1583.55 | 147.1111 | 1 |
| 75 | Unknown                        | 10.2   | 1588.88 | 235.1    | 4 |
| 76 | Unknown                        | 10.238 | 1593.57 | 142.1581 | 4 |
| 77 | Unknown                        | 10.257 | 1595.92 | 117.0769 | 4 |
| 78 | 3-Hydroxy-3-methylglutarate    | 10.399 | 1613.92 | 247.0786 | 1 |
| 79 | Glutamic acid_3TMS             | 10.521 | 1629.72 | 246.1047 | 1 |
| 80 | 4-Hydroxybenzoic acid          | 10.575 | 1636.61 | 98.11    | 1 |
| 81 | Unknown                        | 10.602 | 1640.1  | 117.092  | 4 |
| 82 | Phenylalanine_2TMS             | 10.626 | 1643.17 | 218.084  | 1 |
| 83 | 4-Hydroxyphenylacetic acid     | 10.665 | 1648.28 | 179.100  | 1 |
| 84 | Unknown                        | 10.774 | 1662.36 | 323.1781 | 4 |
| 85 | N-Acetyl aspartic acid_2_major | 10.906 | 1679.35 | 275.1567 | 1 |
| 86 | Asparagine_3TMS                | 10.942 | 1683.97 | 116.1094 | 1 |

|     |                                  |        |         |          |   |
|-----|----------------------------------|--------|---------|----------|---|
| 87  | Arabionose                       | 10.953 | 1685.35 | 103.05   | 1 |
| 88  | Ribose                           | 11.072 | 1700.76 | 103.0844 | 1 |
| 89  | Unknown                          | 11.159 | 1712.59 | 117.1    | 4 |
| 90  | 2-Aminoadipic acid               | 11.263 | 1726.81 | 260.129  | 1 |
| 91  | Xylitol                          | 11.316 | 1733.98 | 217.1    | 1 |
| 92  | Arabitol                         | 11.398 | 1745.14 | 103.1    | 1 |
| 93  | Unknown                          | 11.452 | 1752.56 | 147.15   | 4 |
| 94  | Putrescine                       | 11.494 | 1758.22 | 174.1167 | 1 |
| 95  | Unknown                          | 11.542 | 1764.78 | 116.119  | 4 |
| 96  | Ornithine_3TMS                   | 11.567 | 1768.17 | 174.1125 | 1 |
| 97  | Unknown                          | 11.664 | 1781.42 | 299.0889 | 4 |
| 98  | Glutamine_3TMS                   | 11.702 | 1786.47 | 156.1097 | 1 |
| 99  | O-Phosphoethanolamine            | 11.831 | 1804.32 | 299.0917 | 1 |
| 100 | O-Phosphoethanolamine            | 11.841 | 1805.72 | 100.075  | 1 |
| 101 | Unknown                          | 11.999 | 1828.36 | 147.125  | 4 |
| 102 | Citrulline                       | 12.062 | 1837.32 | 142.1548 | 1 |
| 103 | Isocitric acid+Citric acid       | 12.081 | 1840.01 | 273.1056 | 1 |
| 104 | Myristic acid                    | 12.153 | 1850.3  | 285.22   | 1 |
| 105 | Unknown                          | 12.168 | 1852.47 | 147.1036 | 4 |
| 106 | Unknown                          | 12.202 | 1857.39 | 102.0891 | 4 |
| 107 | Unknown                          | 12.268 | 1866.84 | 217.095  | 4 |
| 108 | 3-Hydroxyanthranilic acid        | 12.409 | 1886.96 | 354.1468 | 1 |
| 109 | Unknown                          | 12.51  | 1901.47 | 218.0844 | 4 |
| 110 | Psicose_2_major+Tagatose_2_major | 12.529 | 1904.38 | 103.0603 | 1 |
| 111 | Sorbose_2                        | 12.543 | 1906.52 | 218.0845 | 1 |
| 112 | Unknown                          | 12.572 | 1910.86 | 327.1042 | 4 |
| 113 | Allantoin                        | 12.587 | 1913.12 | 331.1542 | 1 |
| 114 | Unknown                          | 12.613 | 1916.99 | 210.13   | 4 |
| 115 | Mannose_1_major                  | 12.633 | 1919.96 | 205.0833 | 1 |
| 116 | Glucose_1                        | 12.72  | 1932.96 | 205.0963 | 1 |
| 117 | Lysine_4TMS                      | 12.766 | 1939.97 | 174.1097 | 1 |
| 118 | Histidine_3TMS                   | 12.78  | 1942.04 | 154.1125 | 1 |
| 119 | Galactose_2+Glucose_2            | 12.858 | 1953.68 | 205.0883 | 1 |
| 120 | Tyrosine                         | 12.891 | 1958.73 | 218.0891 | 1 |
| 121 | Mannitol                         | 12.954 | 1968.22 | 205.0857 | 1 |
| 122 | Unknown                          | 12.994 | 1974.12 | 232.0979 | 4 |
| 123 | Glucuronate_1_major              | 13.001 | 1975.19 | 333.1425 | 1 |
| 124 | Galactitol                       | 13.026 | 1979.02 | 217.0917 | 1 |
| 125 | Unknown                          | 13.127 | 1994.1  | 318.1405 | 4 |
| 126 | Glucarate_2_same                 | 13.142 | 1996.33 | 128.1375 | 1 |
| 127 | Unknown                          | 13.201 | 2005.45 | 191.05   | 4 |
| 128 | Galacturonic acid_2_minor        | 13.215 | 2007.75 | 147.1109 | 1 |
| 129 | Unknown                          | 13.277 | 2017.49 | 128.1196 | 4 |
| 130 | Unknown                          | 13.3   | 2021.15 | 227.1483 | 4 |
| 131 | Unknown                          | 13.404 | 2037.4  | 147.1075 | 4 |

|     |                         |        |         |          |   |
|-----|-------------------------|--------|---------|----------|---|
| 132 | Xanthine                | 13.422 | 2040.39 | 353.1286 | 1 |
| 133 | Gluconic acid           | 13.443 | 2043.61 | 147.1167 | 1 |
| 134 | Plamitic acid(16:0)     | 13.468 | 2047.54 | 117.0742 | 1 |
| 135 | Unknown                 | 13.477 | 2048.96 | 143.15   | 4 |
| 136 | Unknown                 | 13.539 | 2058.69 | 205.0765 | 4 |
| 137 | Saccharic acid          | 13.549 | 2060.24 | 333.1293 | 1 |
| 138 | Unknown                 | 13.711 | 2085.82 | 333.1271 | 4 |
| 139 | Uric acid               | 13.965 | 2126.99 | 441.176  | 1 |
| 140 | Unknown                 | 13.974 | 2128.36 | 87.08276 | 4 |
| 141 | Inositol                | 14.002 | 2133.02 | 217.0938 | 1 |
| 142 | Heptadecanic acid(17:0) | 14.085 | 2146.61 | 117.0893 | 1 |
| 143 | Unknown                 | 14.369 | 2193.24 | 232.11   | 4 |
| 144 | Unknown                 | 14.395 | 2197.54 | 174.1281 | 4 |
| 145 | Tryptophan_1TMS         | 14.524 | 2219.61 | 218.081  | 1 |
| 146 | Oleic acid(18:1n-9)     | 14.537 | 2221.8  | 117.075  | 1 |
| 147 | Tryptamine_1            | 14.613 | 2234.91 | 218.0768 | 1 |
| 148 | Stearic acid(17:0)      | 14.673 | 2245.22 | 117.0773 | 1 |
| 149 | Tryptophan_3TMS         | 14.708 | 2251.12 | 202.0852 | 1 |
| 150 | Unknown                 | 14.844 | 2274.58 | 188.1212 | 4 |
| 151 | Unknown                 | 15.047 | 2309.86 | 232.0984 | 4 |
| 152 | Unknown                 | 15.106 | 2320.36 | 204.0842 | 4 |
| 153 | Unknown                 | 15.285 | 2352.42 | 204.063  | 4 |
| 154 | Fructose 6-phosphate_2  | 15.318 | 2358.38 | 315.1136 | 1 |
| 155 | Sorbitol 6-phosphate    | 15.331 | 2360.62 | 387.1433 | 1 |
| 156 | Fructose 6-phosphate_3  | 15.396 | 2372.27 | 387.1552 | 1 |
| 157 | Unknown                 | 15.458 | 2383.42 | 98.0625  | 4 |
| 158 | Unknown                 | 15.483 | 2387.85 | 147.1071 | 4 |
| 159 | Unknown                 | 15.585 | 2406.27 | 232.1018 | 4 |
| 160 | Unknown                 | 15.717 | 2430.75 | 217.0963 | 4 |
| 161 | Unknown                 | 15.844 | 2454.33 | 217.0893 | 4 |
| 162 | Unknown                 | 15.9   | 2464.63 | 217.1    | 4 |
| 163 | Unknown                 | 15.957 | 2475.22 | 318.1317 | 4 |
| 164 | Unknown                 | 16.214 | 2523.78 | 217.0894 | 4 |
| 165 | Unknown                 | 16.252 | 2531.28 | 217.0909 | 4 |
| 166 | Unknown                 | 16.363 | 2552.81 | 217.09   | 4 |
| 167 | Unknown                 | 16.487 | 2576.78 | 98.10333 | 4 |
| 168 | Unknown                 | 16.594 | 2597.53 | 371.3021 | 4 |
| 169 | Adenosine               | 16.956 | 2669.81 | 236.075  | 1 |
| 170 | Sucrose                 | 17.132 | 2705.15 | 361.15   | 1 |
| 171 | Unknown                 | 17.448 | 2770.95 | 98.10625 | 4 |
| 172 | b-Lactose_2_minor       | 17.498 | 2781.38 | 204.0925 | 1 |
| 173 | Unknown                 | 17.58  | 2798.6  | 204.0781 | 4 |
| 174 | Trehalose               | 17.669 | 2817.5  | 361.1672 | 1 |
| 175 | Unknown                 | 17.741 | 2833.05 | 236.05   | 4 |
| 176 | Lactitol                | 17.771 | 2839.48 | 204.079  | 1 |

|     |                          |        |         |          |   |
|-----|--------------------------|--------|---------|----------|---|
| 177 | Unknown                  | 17.965 | 2881.21 | 204.0929 | 4 |
| 178 | Gentiobiose_1_major      | 18.023 | 2893.61 | 204.075  | 1 |
| 179 | Gentiobiose_2_minor      | 18.131 | 2917.23 | 204.0742 | 1 |
| 180 | Melibiose_1_major        | 18.251 | 2944.04 | 361.1625 | 1 |
| 181 | Maltitol                 | 18.261 | 2946.3  | 204.0673 | 1 |
| 182 | Unknown                  | 18.346 | 2965.11 | 98.10455 | 4 |
| 183 | Daidzein                 | 18.391 | 2975.15 | 398.1516 | 1 |
| 184 | Melibiose_2_minor        | 18.405 | 2978.22 | 204.0929 | 1 |
| 185 | Genistein                | 18.456 | 2989.65 | 471.1609 | 1 |
| 186 | Unknown                  | 18.551 | 3010.9  | 105.0964 | 4 |
| 187 | Pyridoxal 5'-phosphate_1 | 18.656 | 3034.59 | 204.0759 | 1 |
| 188 | Unknown                  | 18.707 | 3045.97 | 204.05   | 4 |
| 189 | Galactinol               | 18.845 | 3077.11 | 204.0667 | 1 |
| 190 | Inosine 5'-phosphate     | 19.017 | 3115    | 169.0822 | 1 |
| 191 | Unknown                  | 19.224 | 3158.7  | 98.09531 | 4 |
| 192 | Unknown                  | 19.396 | 3195.08 | 204.0711 | 4 |
| 193 | Unknown                  | 20.239 | 3353.85 | 98.10606 | 4 |
| 194 | Raffinose                | 21.166 | 3502.98 | 361.172  | 1 |
| 195 | Unknown                  | 21.508 | 3550.05 | 98.10645 | 4 |
| 196 | Melezitose               | 21.875 | 3600.37 | 361.1722 | 1 |
| 197 | Unknown                  | 23.174 | 3746.86 | 98.1025  | 4 |
| 198 | Panose_2_major           | 23.56  | 3786.54 | 204.1    | 1 |
| 199 | Panose_1_minor           | 23.637 | 3794.43 | 204.065  | 1 |

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Table S6. List of 17 sensory attributes in Chapter 3

| Category      | No | Attribute      | Description                                                                                                            |
|---------------|----|----------------|------------------------------------------------------------------------------------------------------------------------|
| Color<br>色    | 1  | Golden-brown   | Typical tempe color ranging from light golden brown to dark golden brown (テンペの色。金色のような茶色の強さ)                           |
| Texture<br>質感 | 2  | Compact        | Compactness of bean (high score indicates extremely dense product such as fudge brownie) (豆のコンパクトさ。高いスコアは密度が高いことを意味する) |
|               | 3  | Crispy         | Easy to break into crumbs or pieces associated with potato crisps (ポテトチップスのように簡単に碎ける度合い)                               |
| Aroma<br>香り   | 4  | Beany          | Aroma associated with cooked legumes (調理された豆のような匂い)                                                                    |
|               | 5  | Specific tempe | Intensity of “tempe-specific” aroma (テンペ特有の匂い)                                                                         |
|               | 6  | Alcohol        | Cold, pungent smell associated with alcohol and distilled spirits (アルコールや蒸留酒のような冷たく刺激のある匂い)                            |

|            |    |          |                                                                                                                  |
|------------|----|----------|------------------------------------------------------------------------------------------------------------------|
|            | 7  | Pleasant | Aroma with a good connotation<br>(いい匂い)                                                                          |
|            | 8  | Yeast    | A sour, fermented aromatic commonly associated with yeast<br>(酵母のような酸味のある発酵の匂い)                                  |
|            | 9  | Ammonia  | Pungent described as sharp sensation of nasal mucous membranes associated with vinegar<br>(お酢のような鼻の粘膜を刺激するような匂い) |
| Taste<br>味 | 10 | Sweet    | The fundamental taste factor associated with a sucrose solution<br>(甘味。シヨ糖溶液のような味覚)                              |
|            | 11 | Sour     | A fundamental taste factor of which citric acid in water is typical (酸味。クエン酸のような味覚)                              |
|            | 12 | Salty    | The fundamental taste factor associated with sodium chloride (salt) solution (塩味。塩化ナトリウムのような味覚)                  |
|            | 13 | Bitter   | A fundamental taste factor of which caffeine or quinine is typical (苦                                            |

|               |    |               |                                                                                                            |
|---------------|----|---------------|------------------------------------------------------------------------------------------------------------|
|               |    |               | 味。カフェインまたはキニーネのような味覚)                                                                                      |
|               | 14 | Umami         | The fundamental taste factor of monosodium glutamate is typical (旨味。グルタミン酸ナトリウムのような味覚)                     |
|               | 15 | Astringent    | The drying, puckering sensation on the tongue and other mouth surfaces (舌の表面にしわが寄るようなちくちくする感覚)             |
|               | 16 | Aftertaste    | The lingering taste of the food even after the food is no longer in the mouth (食べ物が口に入らなくなった後も、その味が残ること)   |
| Hedonic<br>嗜好 | 17 | Acceptability | Term to describe acceptance of tempe as an overall value concurrent with the all attributes (テンペとしての総合的な値) |

Table S7. the loading value of metabolites in chapter 3

| Metabolites              | PC1        | PC2         |
|--------------------------|------------|-------------|
| Unknown1                 | 0.074884   | 0.0671606   |
| Unknown2                 | -0.0441616 | 0.00287701  |
| Unknown3                 | -0.0475916 | -0.0463668  |
| Unknown4                 | 0.0173056  | 0.025989    |
| Propyleneglycol          | 0.101662   | 0.0464578   |
| Unknown5                 | 0.0954985  | 0.0666853   |
| 2-Hydroxypyridine        | 0.0987635  | 0.0483053   |
| Unknown6                 | -0.0117112 | -0.0253107  |
| Unknown7                 | 0.0843962  | 0.0980467   |
| Oxalacetic acid+Pyruvate | 0.0362862  | -0.0717626  |
| Unknown8                 | -0.0282288 | -0.0827269  |
| Unknown9                 | -0.0176569 | 0.0261772   |
| Lactic acid              | -0.0161192 | -0.084145   |
| Unknown10                | 0.104555   | 0.0720536   |
| Valine_1TMS_minor        | 0.0414548  | 0.126429    |
| Alanine_2TMS             | -0.0661216 | -0.108739   |
| Unknown11                | 0.0886947  | -0.0790751  |
| Unknown12                | -0.0490655 | 0.0202726   |
| Unknown13                | -0.0338778 | 0.0383474   |
| Leucine_1TMS             | 0.00799297 | 0.0796783   |
| 2-Aminobutyric acid      | 0.0394958  | -0.104753   |
| Isoleucine_1TMS_minor    | 0.052854   | 0.0790683   |
| Unknown14                | 0.112416   | -0.00537143 |

|                             |             |             |
|-----------------------------|-------------|-------------|
| Unknown15                   | -0.0488467  | 0.0354713   |
| Unknown16                   | 0.00675129  | 0.0996325   |
| Unknown17                   | 0.107444    | 0.00145735  |
| Valine_2TMS                 | -0.0279866  | -0.111654   |
| Urea                        | 0.0822307   | 0.113173    |
| Unknown18                   | 0.0539627   | 0.0209265   |
| Unknown19                   | -0.0267412  | -0.129168   |
| Serine_2TMS_minor           | 0.0979964   | 0.0725376   |
| Unknown20                   | 0.0952483   | -0.0142367  |
| 2-Aminoethanol              | 0.065269    | -0.0813076  |
| Leucine_2TMS                | -0.0363939  | -0.135165   |
| Glycerol                    | 0.0814191   | 0.0796869   |
| Nicotinic acid              | 0.105519    | -0.0145924  |
| Threonine_2TMS_minor        | 0.0806591   | 0.0734739   |
| Isoleucine_2TMS_major       | 0.00575315  | -0.150986   |
| Proline_2TMS                | -0.00426131 | -0.128747   |
| Succinic acid(or anhydride) | 0.106502    | -0.0680535  |
| Glycine_3TMS                | 0.0995469   | -0.08126    |
| Glyceric acid               | 0.101162    | -0.0599899  |
| Uracil                      | 0.00842154  | -0.0955921  |
| Fumaric acid                | 0.114563    | -0.00204859 |
| Serine_3TMS_major           | 0.0770649   | -0.0777792  |
| Alanine_3TMS                | -0.0359344  | 0.056958    |
| 3-Pyridyl acetic acid       | -0.0689786  | 0.0261704   |
| Threonine_3TMS_major        | 0.0204638   | -0.0869602  |
| Glutaric acid(or anhydride) | 0.0746278   | -0.101949   |

|                             |             |             |
|-----------------------------|-------------|-------------|
| Thymine                     | 0.11361     | -0.0103808  |
| Unknown21                   | 0.0989828   | 0.05591     |
| b-Alanine_3TMS              | 0.100565    | -0.0418591  |
| Unknown22                   | -0.0155942  | 0.0687487   |
| Unknown23                   | 0.0525835   | -0.00306105 |
| Unknown24                   | -0.00540162 | 0.0709282   |
| Unknown25                   | 0.110401    | 0.0271456   |
| Unknown26                   | -0.0463437  | 0.0435007   |
| Malic acid                  | 0.113893    | -0.00447996 |
| Unknown27                   | 0.109335    | 0.0302272   |
| Unknown28                   | 0.012092    | 0.0259947   |
| Threitol                    | 0.110071    | -0.0172579  |
| Unknown29                   | -0.00735851 | 0.0668417   |
| Meso erythritol             | 0.0758011   | -0.00726685 |
| Aspartic acid_3TMS          | 0.0990969   | -0.0674726  |
| Pyroglutamic acid_2TMS      | 0.110785    | 0.0351269   |
| Unknown30                   | 0.0962019   | 0.0560925   |
| Hydroxyproline              | 0.0640018   | -0.0327062  |
| 4-Aminobutyric acid         | -0.104381   | 0.0123703   |
| Unknown31                   | 0.083174    | 0.0918579   |
| Unknown32                   | -0.0380176  | 0.0351358   |
| Unknown33                   | -0.0418751  | 0.0597657   |
| Threonic acid               | 0.0333806   | 0.0739615   |
| Unknown34                   | -0.0291854  | 0.0245765   |
| $\alpha$ -ketoglutaric acid | 0.089662    | -0.07162    |
| Unknown35                   | -0.0125037  | 0.0261978   |

|                                |            |              |
|--------------------------------|------------|--------------|
| Unknown36                      | 0.027729   | 0.0801078    |
| Unknown37                      | 0.112713   | -0.00977891  |
| 3-Hydroxy-3-methylglutarate    | 0.0805872  | -0.0932993   |
| Glutamic acid_3TMS             | 0.0867848  | -0.0833416   |
| 4-Hydroxybenzoic acid          | 0.0952155  | 0.0206659    |
| Unknown38                      | 0.109093   | 0.0318558    |
| Phenylalanine_2TMS             | -0.0876451 | -0.0734112   |
| 4-Hydroxyphenylacetic acid     | 0.0533802  | 0.134547     |
| Unknown39                      | -0.0227745 | -0.0716286   |
| N-Acetyl aspartic acid_2_major | 0.104541   | 0.00890466   |
| Asparagine_3TMS                | 0.0947869  | -0.0164377   |
| Arabionose                     | 0.072587   | 0.0153448    |
| Ribose                         | 0.102825   | 0.0116452    |
| Unknown40                      | 0.01379    | 0.0500545    |
| 2-Aminoadipic acid             | 0.0766723  | -0.108295    |
| Xylitol                        | 0.0825326  | -0.000399937 |
| Arabitol                       | -0.0503536 | 0.0255262    |
| Unknown41                      | 0.0163624  | -0.0157269   |
| Putrescine                     | 0.110538   | -0.0272899   |
| Unknown42                      | 0.086202   | -0.0577688   |
| Ornithine_3TMS                 | 0.0800102  | 0.0581417    |
| Unknown43                      | -0.0503386 | 0.0477692    |
| Glutamine_3TMS                 | 0.101935   | -0.0134391   |
| O-Phosphoethanolamine          | 0.0599087  | -0.128538    |
| Unknown44                      | 0.0851312  | -0.0262847   |
| Citrulline                     | 0.0926846  | 0.0189182    |

|                                  |             |              |
|----------------------------------|-------------|--------------|
| Isocitric acid+Citric acid       | 0.102049    | -0.0554938   |
| Myristic acid                    | 0.00985029  | 0.0185252    |
| Unknown45                        | 0.0665123   | -0.0435615   |
| Unknown46                        | 0.109416    | 0.0238461    |
| Unknown47                        | -0.0481013  | 0.108371     |
| 3-Hydroxyanthranilic acid        | 0.0826137   | 0.101727     |
| Unknown48                        | 0.108974    | 0.0371069    |
| Psicose_2_major+Tagatose_2_major | 0.0430137   | 0.0461847    |
| Sorbose_2                        | 0.108483    | 0.0286952    |
| Unknown49                        | 0.0295379   | -0.000852844 |
| Allantoin                        | 0.0986657   | 0.038373     |
| Unknown50                        | 0.106184    | -0.0159095   |
| Mannose_1_major                  | 0.0848689   | -0.0175521   |
| Glucose_1                        | 0.0741461   | 0.000252646  |
| Lysine_4TMS                      | -0.0866146  | 0.00997879   |
| Histidine_3TMS                   | 0.0996726   | -0.043509    |
| Galactose_2+Glucose_2            | 0.0753865   | -0.013387    |
| Tyrosine                         | -0.0988206  | -0.0275591   |
| Mannitol                         | 0.0600553   | -0.039837    |
| Unknown51                        | 0.0498467   | 0.101303     |
| Glucuronate_1_major              | 0.0895802   | -0.00183717  |
| Galactitol                       | 0.0986069   | 0.0634371    |
| Unknown52                        | 0.0592494   | 0.0109615    |
| Glucarate_2_same                 | 0.112802    | 0.00245617   |
| Unknown53                        | -0.00768223 | 0.108971     |
| Galacturonic acid_2_minor        | -0.0202753  | 0.162091     |

|                         |             |             |
|-------------------------|-------------|-------------|
| Unknown54               | 0.112385    | 0.00834248  |
| Unknown55               | 0.0843227   | 0.0820644   |
| Unknown56               | 0.0481469   | 0.108598    |
| Xanthine                | -0.0736782  | -0.00928025 |
| Gluconic acid           | 0.0454853   | -0.128841   |
| Plamitic acid(16:0)     | -0.0178686  | 0.047612    |
| Unknown57               | 0.0421426   | 0.0391757   |
| Unknown58               | 0.0185036   | 0.0940186   |
| Saccharic acid          | -0.043264   | 0.144736    |
| Unknown59               | -0.0123362  | 0.160868    |
| Uric acid               | 0.0717262   | -0.0794395  |
| Unknown60               | -0.00973617 | 0.0453373   |
| Inositol                | -0.0605726  | -0.0289557  |
| Heptadecanic acid(17:0) | -0.0147317  | -0.00718061 |
| Unknown61               | -0.100483   | 0.0476759   |
| Unknown62               | 0.107067    | -0.0189723  |
| Tryptophan_1TMS         | -0.0590788  | 0.0493963   |
| Oleic acid(18:1n-9)     | -0.034996   | 0.0155379   |
| Tryptamine_1            | -0.103165   | -0.00619396 |
| Stearic acid(17:0)      | -0.0171187  | 0.0431245   |
| Tryptophan_3TMS         | -0.0347469  | -0.10464    |
| Unknown63               | -0.0596413  | 0.0133585   |
| Unknown64               | 0.0832569   | 0.0553229   |
| Unknown65               | 0.0908322   | 0.0805629   |
| Unknown66               | -0.0262948  | 0.130574    |
| Fructose 6-phosphate_2  | -0.081162   | 0.0210728   |

|                        |            |            |
|------------------------|------------|------------|
| Sorbitol 6-phosphate   | -0.0653683 | 0.00346552 |
| Fructose 6-phosphate_3 | -0.0769517 | 0.00864245 |
| Unknown67              | 0.0365699  | 0.0668601  |
| Unknown68              | 0.00916309 | 0.116465   |
| Unknown69              | -0.0858412 | 0.0426605  |
| Unknown70              | 0.102382   | 0.0851353  |
| Unknown71              | 0.0344542  | 0.0910286  |
| Unknown72              | 0.0590263  | -0.0804118 |
| Unknown73              | -0.0900712 | 0.00811472 |
| Unknown74              | 0.0492373  | -0.0372073 |
| Unknown75              | 0.0766892  | 0.0392311  |
| Unknown76              | 0.0432382  | -0.0426053 |
| Unknown77              | 0.0586483  | 0.0812312  |
| Unknown78              | -0.0247812 | 0.0778652  |
| Adenosine              | -0.0478206 | 0.0878903  |
| Sucrose                | -0.0408071 | 0.0496774  |
| Unknown79              | 0.0464897  | 0.0529729  |
| b-Lactose_2_minor      | 0.0771206  | 0.0376281  |
| Unknown80              | 0.0748469  | 0.0324829  |
| Trehalose              | 0.113243   | -0.0165763 |
| Unknown81              | 0.0406675  | -0.154623  |
| Lactitol               | -0.0785853 | 0.0996002  |
| Unknown82              | 0.0506613  | -0.106704  |
| Gentiobiose_1_major    | 0.0144944  | -0.133012  |
| Gentiobiose_2_minor    | -0.0472295 | 0.125035   |
| Melibiose_1_major      | 0.0410829  | 0.110496   |

|                          |            |             |
|--------------------------|------------|-------------|
| Maltitol                 | 0.00323684 | 0.150391    |
| Unknown83                | 0.0590256  | 0.0645801   |
| Daidzein                 | 0.0651569  | -0.0325084  |
| Melibiose_2_minor        | 0.00756832 | 0.139653    |
| Genistein                | 0.0318782  | -0.0487793  |
| Unknown84                | 0.00897593 | 0.0432046   |
| Pyridoxal 5'-phosphate_1 | -0.0745133 | 0.111965    |
| Unknown85                | 0.0530317  | 0.0662099   |
| Galactinol               | -0.100662  | 0.0710285   |
| Inosine 5'-phosphate     | 0.103593   | -0.00245665 |
| Unknown86                | 0.0390076  | 0.0445108   |
| Unknown87                | -0.0941299 | 0.0643949   |
| Unknown88                | 0.0438535  | 0.0745719   |
| Raffinose                | -0.0212345 | 0.124395    |
| Unknown89                | 0.0505395  | 0.0661707   |
| Melezitose               | 0.0404501  | -0.0263326  |
| Unknown90                | 0.0369639  | 0.044789    |
| Panose_2_major           | 0.016314   | -0.155524   |
| Panose_1_minor           | -0.0660165 | -0.0916436  |

---

Table S8 VIP metabolites (Score > 1.00) for Golden-brown attributes in Chapter 3

| G. Brown                       |           |
|--------------------------------|-----------|
| Metabolite name                | VIP Score |
| Thymine                        | 1.48883   |
| Fumaric acid                   | 1.46473   |
| Trehalose                      | 1.45601   |
| Ribose                         | 1.45287   |
| Malic acid                     | 1.43187   |
| Threitol                       | 1.42753   |
| Putrescine                     | 1.40818   |
| Nicotinic acid                 | 1.40449   |
| 4-Aminobutyric acid            | 1.38806   |
| Glucarate_2_same               | 1.38323   |
| Pyroglutamic acid_2TMS         | 1.35743   |
| Succinic acid(or anhydride)    | 1.33448   |
| Tryptamine_1                   | 1.33333   |
| Sorbose_2                      | 1.33068   |
| Isocitric acid+Citric acid     | 1.31313   |
| Inosine 5'-phosphate           | 1.30324   |
| Hydroxyproline                 | 1.30072   |
| Galactinol                     | 1.29808   |
| N-Acetyl aspartic acid_2_major | 1.28352   |
| Galactitol                     | 1.27693   |
| b-Alanine_3TMS                 | 1.2763    |
| Glyceric acid                  | 1.27293   |

|                                  |         |
|----------------------------------|---------|
| Aspartic acid_3TMS               | 1.26654 |
| Glutamine_3TMS                   | 1.26491 |
| Allantoin                        | 1.2615  |
| Phenylalanine_2TMS               | 1.26008 |
| Histidine_3TMS                   | 1.25182 |
| Glycine_3TMS                     | 1.2478  |
| 2-Hydroxypyridine                | 1.23125 |
| Tyrosine                         | 1.22506 |
| Propyleneglycol                  | 1.21996 |
| Serine_2TMS_minor                | 1.17798 |
| 3-Pyridyl acetic acid            | 1.17583 |
| Asparagine_3TMS                  | 1.1621  |
| Urea                             | 1.15281 |
| 4-Hydroxybenzoic acid            | 1.14615 |
| b-Lactose_2_minor                | 1.14562 |
| Glutamic acid_3TMS               | 1.13517 |
| $\alpha$ -ketoglutaric acid      | 1.13    |
| 3-Hydroxyanthranilic acid        | 1.11944 |
| Citrulline                       | 1.09723 |
| Inositol                         | 1.09041 |
| Lysine_4TMS                      | 1.08609 |
| Glycerol                         | 1.05609 |
| Mannose_1_major                  | 1.03627 |
| Glucuronate_1_major              | 1.03268 |
| Psicose_2_major+Tagatose_2_major | 1.01616 |

|            |         |
|------------|---------|
| Xanthine   | 1.01078 |
| Daidzein   | 1.00925 |
| Arabionose | 1.00753 |
| Xylitol    | 1.00752 |

---

Table S9. VIP metabolites (Score > 1.00) for pleasant sensory attributes in Chapter 3

| Pleasant                    |           |
|-----------------------------|-----------|
| Metabolite name             | VIP Score |
| Succinic acid(or anhydride) | 1.45801   |
| Glycine_3TMS                | 1.43538   |
| Trehalose                   | 1.41656   |
| Hydroxyproline              | 1.41091   |
| Galactinol                  | 1.40638   |
| Thymine                     | 1.40569   |
| Putrescine                  | 1.38638   |
| Aspartic acid_3TMS          | 1.38063   |
| Fumaric acid                | 1.37607   |
| Isocitric acid+Citric acid  | 1.37034   |
| Malic acid                  | 1.36323   |
| Glyceric acid               | 1.35985   |
| Threitol                    | 1.32852   |
| b-Alanine_3TMS              | 1.32585   |
| Glucarate_2_same            | 1.31017   |
| 4-Aminobutyric acid         | 1.29645   |
| Glutamic acid_3TMS          | 1.29398   |
| Histidine_3TMS              | 1.2853    |
| Ribose                      | 1.28101   |
| Pyroglutamic acid_2TMS      | 1.25558   |
| Nicotinic acid              | 1.25509   |
| $\alpha$ -ketoglutaric acid | 1.25509   |

|                                |         |
|--------------------------------|---------|
| Glutamine_3TMS                 | 1.23955 |
| Sorbose_2                      | 1.23606 |
| 2-Aminoadipic acid             | 1.23579 |
| Inosine 5'-phosphate           | 1.23233 |
| 3-Hydroxy-3-methylglutarate    | 1.22737 |
| N-Acetyl aspartic acid_2_major | 1.22315 |
| Glutaric acid(or anhydride)    | 1.2184  |
| Tryptamine_1                   | 1.21256 |
| 3-Pyridyl acetic acid          | 1.1862  |
| Lactitol                       | 1.17441 |
| Pyridoxal 5'-phosphate_1       | 1.15435 |
| Asparagine_3TMS                | 1.13261 |
| Phenylalanine_2TMS             | 1.11812 |
| Allantoin                      | 1.11159 |
| Propyleneglycol                | 1.1108  |
| Serine_3TMS_major              | 1.10186 |
| Tyrosine                       | 1.08963 |
| 2-Hydroxypyridine              | 1.0811  |
| Serine_2TMS_minor              | 1.07774 |
| O-Phosphoethanolamine          | 1.07357 |
| Galactitol                     | 1.06643 |
| 4-Hydroxybenzoic acid          | 1.04327 |
| Mannose_1_major                | 1.03608 |
| Uric acid                      | 1.02971 |
| Citrulline                     | 1.02469 |

|             |         |
|-------------|---------|
| Inositol    | 1.02129 |
| Lysine_4TMS | 1.01162 |

---

Table S10. VIP metabolites (Score > 1.00) for ammonia smell sensory attributes in  
Chapter 3

| Ammonia                        |           |
|--------------------------------|-----------|
| Metabolite name                | VIP Score |
| Thymine                        | 1.47144   |
| Fumaric acid                   | 1.45346   |
| Ribose                         | 1.43929   |
| Trehalose                      | 1.43696   |
| Malic acid                     | 1.41812   |
| Threitol                       | 1.41074   |
| Putrescine                     | 1.3876    |
| Nicotinic acid                 | 1.38311   |
| 4-Aminobutyric acid            | 1.36819   |
| Glucarate_2_same               | 1.36711   |
| Pyroglutamic acid_2TMS         | 1.35047   |
| Tryptamine_1                   | 1.3254    |
| Sorbose_2                      | 1.31899   |
| Galactitol                     | 1.31321   |
| Succinic acid(or anhydride)    | 1.3062    |
| Inosine 5'-phosphate           | 1.29129   |
| Isocitric acid+Citric acid     | 1.27888   |
| Phenylalanine_2TMS             | 1.27357   |
| Allantoin                      | 1.2697    |
| Hydroxyproline                 | 1.26856   |
| N-Acetyl aspartic acid_2_major | 1.26444   |

|                             |         |
|-----------------------------|---------|
| Galactinol                  | 1.26161 |
| b-Alanine_3TMS              | 1.25209 |
| 2-Hydroxypyridine           | 1.24938 |
| Glutamine_3TMS              | 1.24395 |
| Glyceric acid               | 1.24385 |
| Aspartic acid_3TMS          | 1.23452 |
| Propyleneglycol             | 1.23337 |
| Tyrosine                    | 1.2322  |
| Urea                        | 1.23107 |
| Histidine_3TMS              | 1.22854 |
| Glycine_3TMS                | 1.22214 |
| Serine_2TMS_minor           | 1.19258 |
| 3-Hydroxyanthranilic acid   | 1.17739 |
| b-Lactose_2_minor           | 1.16576 |
| 4-Hydroxybenzoic acid       | 1.1479  |
| 3-Pyridyl acetic acid       | 1.13985 |
| Asparagine_3TMS             | 1.13842 |
| Glutamic acid_3TMS          | 1.08686 |
| $\alpha$ -ketoglutaric acid | 1.08504 |
| Inositol                    | 1.08046 |
| Citrulline                  | 1.07933 |
| Glycerol                    | 1.0746  |
| Alanine_2TMS                | 1.07315 |
| Lysine_4TMS                 | 1.07157 |
| Melibiose_1_major           | 1.06877 |

|                                  |         |
|----------------------------------|---------|
| 4-Hydroxyphenylacetic acid       | 1.05511 |
| Psicose_2_major+Tagatose_2_major | 1.0198  |
| Glucuronate_1_major              | 1.01479 |
| Mannose_1_major                  | 1.01452 |
| Arabionose                       | 1.01028 |
| Xylitol                          | 1.00786 |

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Table S11. VIP metabolites (Score > 1.00) for aftertaste sensory attributes in Chapter 3

| Aftertaste                  |           |
|-----------------------------|-----------|
| Metabolite name             | VIP Score |
| Succinic acid(or anhydride) | 1.44881   |
| Trehalose                   | 1.42537   |
| Glycine_3TMS                | 1.41919   |
| Thymine                     | 1.41888   |
| Hydroxyproline              | 1.40546   |
| Galactinol                  | 1.40285   |
| Putrescine                  | 1.39281   |
| Fumaric acid                | 1.39131   |
| Malic acid                  | 1.37632   |
| Aspartic acid_3TMS          | 1.37495   |
| Isocitric acid+Citric acid  | 1.37102   |
| Glyceric acid               | 1.35558   |
| Threitol                    | 1.3419    |
| Glucarate_2_same            | 1.32451   |
| b-Alanine_3TMS              | 1.3206    |
| 4-Aminobutyric acid         | 1.30969   |
| Ribose                      | 1.29951   |
| Glutamic acid_3TMS          | 1.29104   |
| Histidine_3TMS              | 1.28502   |
| Pyroglutamic acid_2TMS      | 1.27719   |
| Nicotinic acid              | 1.27087   |
| Sorbose_2                   | 1.25654   |

|                                |         |
|--------------------------------|---------|
| $\alpha$ -ketoglutaric acid    | 1.25342 |
| Inosine 5'-phosphate           | 1.24547 |
| Glutamine_3TMS                 | 1.24412 |
| N-Acetyl aspartic acid_2_major | 1.23451 |
| Tryptamine_1                   | 1.22973 |
| 2-Aminoadipic acid             | 1.22873 |
| 3-Hydroxy-3-methylglutarate    | 1.21367 |
| Glutaric acid(or anhydride)    | 1.20992 |
| 3-Pyridyl acetic acid          | 1.19093 |
| Lactitol                       | 1.16436 |
| Phenylalanine_2TMS             | 1.14318 |
| Asparagine_3TMS                | 1.13578 |
| Pyridoxal 5'-phosphate_1       | 1.13517 |
| Allantoin                      | 1.13469 |
| Propyleneglycol                | 1.13406 |
| Tyrosine                       | 1.11143 |
| Serine_2TMS_minor              | 1.10698 |
| 2-Hydroxypyridine              | 1.1046  |
| Galactitol                     | 1.09032 |
| Serine_3TMS_major              | 1.08282 |
| 4-Hydroxybenzoic acid          | 1.05734 |
| O-Phosphoethanolamine          | 1.04156 |
| Citrulline                     | 1.03914 |
| Mannose_1_major                | 1.0343  |
| Uric acid                      | 1.03292 |

|             |         |
|-------------|---------|
| Inositol    | 1.03178 |
| Lysine_4TMS | 1.02234 |
| Daidzein    | 1.00404 |

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Table S12. VIP metabolites (Score > 1.00) for acceptability sensory attributes in  
Chapter 3

| Acceptability               |           |
|-----------------------------|-----------|
| Metabolite name             | VIP Score |
| Thymine                     | 1.49242   |
| Trehalose                   | 1.46836   |
| Fumaric acid                | 1.46148   |
| Malic acid                  | 1.43603   |
| Ribose                      | 1.42777   |
| Threitol                    | 1.42577   |
| Putrescine                  | 1.42474   |
| Nicotinic acid              | 1.39666   |
| 4-Aminobutyric acid         | 1.39467   |
| Glucarate_2_same            | 1.39449   |
| Succinic acid(or anhydride) | 1.38529   |
| Isocitric acid+Citric acid  | 1.3756    |
| Galactinol                  | 1.37481   |
| Pyroglutamic acid_2TMS      | 1.36627   |
| Hydroxyproline              | 1.36604   |
| Sorbose_2                   | 1.34829   |
| Aspartic acid_3TMS          | 1.33362   |
| Glyceric acid               | 1.32628   |
| Tryptamine_1                | 1.31764   |
| Glycine_3TMS                | 1.3063    |
| Inosine 5'-phosphate        | 1.30392   |

|                                |         |
|--------------------------------|---------|
| b-Alanine_3TMS                 | 1.29679 |
| N-Acetyl aspartic acid_2_major | 1.29605 |
| Histidine_3TMS                 | 1.28112 |
| Glutamine_3TMS                 | 1.27633 |
| Glutamic acid_3TMS             | 1.26229 |
| $\alpha$ -ketoglutaric acid    | 1.24142 |
| 3-Pyridyl acetic acid          | 1.23509 |
| Phenylalanine_2TMS             | 1.22783 |
| Allantoin                      | 1.22393 |
| Propyleneglycol                | 1.21244 |
| Tyrosine                       | 1.19486 |
| Serine_2TMS_minor              | 1.19047 |
| 2-Hydroxypyridine              | 1.18672 |
| Galactitol                     | 1.17613 |
| Asparagine_3TMS                | 1.17468 |
| 2-Aminoadipic acid             | 1.13499 |
| 4-Hydroxybenzoic acid          | 1.1261  |
| Citrulline                     | 1.12476 |
| Glutaric acid(or anhydride)    | 1.12261 |
| Daidzein                       | 1.08975 |
| Lysine_4TMS                    | 1.08909 |
| Inositol                       | 1.0878  |
| 3-Hydroxy-3-methylglutarate    | 1.08307 |
| Glycerol                       | 1.06979 |
| Uric acid                      | 1.06966 |

|                           |         |
|---------------------------|---------|
| Threonine_2TMS_minor      | 1.06602 |
| Lactitol                  | 1.06279 |
| Xanthine                  | 1.05428 |
| Glucuronate_1_major       | 1.04529 |
| Mannose_1_major           | 1.04514 |
| 3-Hydroxyanthranilic acid | 1.02492 |
| Galactose_2+Glucose_2     | 1.00153 |

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