INTRODUCTION

Taisui, shaped and touched like meat or jelly, was named because of its morphological characteristics that are similar to the description of “Taisui” in ancient China. Firstly, Taisui being discovered in Shaanxi Province in 1992, China (Wang et al., 2017). Until 2013, Taisui had been reported 228 times. Among them, 98.57% of Taisui were accidentally discovered (Wang & Wang, 2014).

Taisui is usually considered as a complex of bacteria, fungi, and myxomycete (Sui, 2019). Yet, the dominant microbes in Taisui from

Abstract

Taisui, a special substance occasionally found in China, can now be artificially cultured. In order to evaluate the safety of an artificially cultured Taisui (acTS) and develop it into fermented, functional food or oral liquid, the macronutrients, trace elements, microbial community, and extracellular metabolites of Taisui have been investigated in this study. Results showed that the concentrations of total carbohydrates, protein, fat, total ash, and moisture of wet acTS were 2.13 g/100 g, 0.13 g/100 g, 0.07 g/100 g, 0.04 g/100 g, and 88.3%, respectively. The concentrations of top three trace elements of K, Ca, and P are 1,424.92 mg/kg, 159.96 mg/kg, and 67.89 mg/kg, respectively. Proteobacteria, Euryarchaeota, and Ascomycota were the three most abundant genera of bacteria; Methanosaeta, Methanosphaera, and Natronomonas, the most abundant genera of archaea; Zygosaccharomyces, Mortierella, and Fusarium, the most abundant genera of fungi. There were 311 metabolites increased in acTS. Most of the metabolites are beneficial to human. These metabolites can be contributed to microbes in acTS. In conclusion, acTS is not a good source of macronutrients and of trace elements, while the safeness of some microorganisms in acTS is also unknown. Nevertheless, it still provides some probiotics and beneficial metabolites for human. It is thus possible to develop acTS into foods when the safety of each microorganism is proved.

KEYWORDS

chemical components, metabolomics, microbial community, Taisui

1 | INTRODUCTION

Taisui, shaped and touched like meat or jelly, was named because of its morphological characteristics that are similar to the description of “Taisui” in ancient China. Firstly, Taisui being discovered in Shaanxi Province in 1992, China (Wang et al., 2017). Until 2013, Taisui had been reported 228 times. Among them, 98.57% of Taisui were accidentally discovered (Wang & Wang, 2014).

Taisui is usually considered as a complex of bacteria, fungi, and myxomycete (Sui, 2019). Yet, the dominant microbes in Taisui from

Chen and Zheng contributed equally to this paper.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2021 The Authors. Food Science & Nutrition published by Wiley Periodicals LLC
various sources differ. For example, Klebsiella oxytoca and Ralstonia eutropha were both found by the plate-culturing and noncultur- ing methods (Wang, 2007), whereas Pseudomonas fluorescens and Brevundimonas mediterranea were only detected from another Taisui sample (Tong et al., 2018). Of fungi, Candida and Rhodotorula mucilaginosa were identified by 18S rDNA sequencing method (Lin et al., 2013), whereas Acremonium and Trichoderma were the dominant fungi of another type of Taisui (Dai, 2007). In terms of myxomycete, Didymium verrucosporum and Diderma deplanatum have been successfully isolated using either corn meal-agar or oat meal-agar method (Dai, 2007). Moreover, archaea have also been found in Taisui and the dominant archaea were Methanobacterium, Methanobrevibacter, Methanosphaera (Wang & Wang, 2017). In ad- dition, studies have investigated Taisui from the perspective of its chemical composition. Polyvinyl alcohol has been found as the main component of meat-like Taisui, whereas polyacrylic acid or polyvinyl alcohol was the main component of jelly-like Taisui (Li et al., 2020; Zheng & Dong, 2010). Some researchers believe that Taisui may have healthy benefits such as regulating immunity, inhibiting tumors, de- laying aging, and eliminating fatigue. Within Taisui, PQQ (pyrrolo- quinoline quinone), nucleic acids, trace elements etc., have also been found as effective ingredients (Wang, 2018). In order to obtain the functional substances secreted from Taisui, Taisui has now been suc- cessfully artificially cultured, some of which are already on the mar - ket. For example, Lactobacillus and Aspergillus, isolated from Taisui (Dai, 2007; Han et al., 2018), can be potentially used in the brewing and soy sauce industry (Fang et al., 2006).

The chemical and microbial compositions of Taisui vary with the source of Taisui (Li et al., 2015; Wang, 2018). Consequently, the chemical compositions, total carbohydrates, protein, fat, total ash, moisture, and trace elements of Taisui cultured in honey solution have been investigated, as well as the microbial structure and extra - cellular metabolites of its bacteria, archaea, and fungi in this manu - script. Its aim is to fully understand the safeness of acTS products.

2 | MATERIALS AND METHODS

2.1 | Material

The mixture of Taisui and honey solution (Figure 1) was kindly gifted by Guangzhou KingCell Co., Ltd. Taisui sample is light yellow, cream, and transparent. It is cultured in honey solution at room temperature (about 25°C) with a ratio of honey to water of 6:1 (v/v).

2.2 | Chemical components analysis

Wet acTS was washed by distilled water and then was homogenized in a pulverizer. All tests were performed in triplicate.

The concentrations of total carbohydrates, protein, fat, total ash, moisture, and trace elements were determined by phenol-sulfuric acid method (Albalasmeh et al., 2013), Kjeldahl method (Oftedal et al., 2014), acid hydrolysis method (Lakshanasomya et al., 2011), burning gravimetric method (Oellig et al., 2020), oven drying method (Chiachung, 2003), and ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry) method (Bitter et al., 2020).

2.3 | Microbial composition of bacteria, archaea and fungi

Due to the difference in appearance, we divided the artificially cul - tured Taisui solution into three parts: the upper, the lower, and the medium part (denoted as group U, group L, group M, respectively). Group U and group L were washed by 75% ethanol solutions and distilled water. All tests were performed in triplicate.

DNA from the three groups was extracted using MN NucleoSpin 96 Soil Kit (Macherey-Nagel). Sequencing was analyzed on the Illumina Mi Seq platform (Illumina MiSeq). The sequences were clustered at a similarity level of 97% (USEARCH, version 10.0) (Edgar, 2013), and the OTUs were filtered with 0.005% of the number of all sequences as a threshold (Bokulich et al., 2013). Microorganisms with less than 0.1% of RA were classified into “Others.”

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) software (version1.0.0) was used to predict functional genes composition. The obtained OTU was

FIGURE 1 Artificially cultured jelly-like Taisui
standardized. According to the unique greengene id corresponding to each OTU, the KEGG family information and the abundance of KEGG were obtained. The abundance of each type of function was obtained from KEGG database.

2.4 | Metabolomics analysis

Cultured Taisui honey solution was set as culture group (denoted as group Cul), and the uncultured Taisui honey solution was set as control group (denoted as group Con). All tests were performed in sextuplicate.

Samples were mixed (v/v, 1:5) with 80% methanol aqueous solution and were collected for analysis using a Thermo Scientific Vanquish UHPLC system with a Thermo Hyperil Gold (C18) column coupled with a Mass Spectrometer detector Q Exactive HF-X (Thermo) (Dunn et al., 2011). Compared with mzCloud database, the data recognition and quantitative results were obtained. Metabolites were determined as compounds with the Variable Importance in Projection (VIP) value \(>1\) and \(p<.05\) and Fold Change value (FC) \(>2\) or \(<0.5\) (Svenja et al., 2016).

2.5 | Statistical analysis

Statistical analysis was performed with SPSS software (IBM, version 22.0). Results were presented as mean ± standard deviation. Two-tailed T tests were conducted to compare the difference among different groups. Pearson correlation was performed with SPSS software (IBM, version 22.0), and heat maps were performed with Origin (OriginLab, version 2018). Statistical significance was set at a \(p\) value < .05.

3 | RESULTS

3.1 | Potential macronutrients and trace elements

As shown in Tables 1 and 2, the total content of carbohydrate was 2.13 g/100 g and was the main nutrient of wet acTS compared with other macronutrient. The protein and fat contents were 0.13 g/100 g and 0.07 g/100 g, respectively. Total ash was the lowest component of solid with a number of 0.04 g/100 g. The moisture concentration was the highest with a number of 88.3%. In total, 15 kinds of trace elements were detected. K was the most abundant (1,424.92 mg/kg), followed by Ca, P, Fe, Mg, and Al within 11–160 mg/kg. The concentrations of macronutrients and trace elements mentioned above were expressed as wet weight.

| Carbohydrate g/100 g | Protein g/100 g | Fat g/100 g | Total ash g/100 g | Moisture % |
|----------------------|----------------|-------------|-------------------|------------|
| 2.13 ± 0.02          | 0.13 ± 0.00    | 0.07 ± 0.00 | 0.04 ± 0.00       | 88.30 ± 0.00 |

3.2 | Microbial composition of bacteria, archaea, and fungi

3.2.1 | OTU, alpha-diversity indices and Venn diagrams

The number of OTU and the alpha diversity indices are shown in Table 3. The OTU numbers and alpha diversity indices indicated that no significant differences were found between group U, L, and M in terms of bacteria and archaea. For fungi, the OTU number, Shannon index, ACE index, and Chao1 index of group L were significantly higher than those of other two groups, and Simpson index was significantly lower than those of other two groups.

The Venn diagram indicated the shared OTUs and unique OTUs of bacteria, archaea, and fungi respectively. In the Venn diagram, there are 1,411 shared OTUs in bacteria, 15 shared OTUs in archaea and 186 shared OTUs in fungi. The shared OTUs accounted for large part of OTUs (Figure S1).

3.2.2 | Composition of microbiota at the phylum level

The microbial community structure of bacteria, archaea, and fungi at the phylum level was presented in Figure 2, and the corresponding relative abundance (RA) was listed in Table S1.

The RA of Proteobacteria was the highest in L, U, and M groups, accounting for 43.10%, 38.73%, and 41.70%. Followed by Chloroflexi, Firmicutes, Acidobacteria, Actinobacteria, and Nitrospirae, these phyla of bacteria were the micro part of bacteria and their RAs were 15%–4%.

The dominant phylum of archaea among 3 groups was Euryarchaeota (>88%), while Crenarchaeota and Diapherotrites only accounted for a low ratio of the community < 10%, respectively.

However, only Ascomycota was predominant among fungi with higher than 46% RA among 3 groups. The RA of Rozellomycota was 19.09% in group U. The RA of Basidiomycota and Mortierellomycota was 7.47% and 3.45% in group U, respectively, and was 6.05% and 2.09% in group M. The RA of Basidiomycota only accounted for 1.77% in group L.

3.2.3 | Composition of microbiota at the genus level

The microbial community structure of bacteria, archaea, and fungi at the genus level was presented in Figure 3 while the top 10 relative abundance (RA) was listed in Table S2.
The dominant bacterial genera of Taisui community were uncultured_bacterium_f_Anaerolineaceae, Alcaligenes, Ochrobactrum, uncultured_bacterium_c_Thermosulfovibrio, and uncultured_bacterium_c_Subgroup_6. The RA of all these genera was about 22%. The RA of uncultured_bacterium_f_Steroidobacteraceae, Methyloptena, Lactobacillus, Ralstonia, and uncultured_bacterium_f_SCI-84 together was less than 14% in the three groups.

* Methanoseta and Methanospirillum were the predominant genera in group L with a RA of 32.03% and 20.63%, respectively. Methanoseta and Natronomonas were the dominant genera in group U with a RA of 30.11% and 23.75%, respectively, and were also the dominant genera in group M, and their RAs were 35.35% and 20.40%. Uncultured_bacterium_c_Bathyarchaeia, Methanobacterium, Methanobrevibacter, Methanosarcina, Methanolinea, and Methanomassiliicoccus were also the major part of archaea community.

* Zygosaccharomyces was the most abundant genus among three groups with RA of 63.17%, 8.23%, and 66.61%. Followed by the genus of fungi Plectosphaerella, Fusarium, Cladosporium, Mortierella, Candida, Penicillium, Chaetomium, and Pseudopithomyces. However, the RA of these genera of fungi only accounted for about 3.33%–0.20%.

### 3.2.4 Functional genes prediction

Based on KEGG database, PICRUSt analysis is used to predict functional genes composition by comparing species composition obtained from the sequencing data. As shown in Figure 4, it was found that the functional genes of Metabolism was the most dominant category. The KEGG level 2 result showed that group U, L, and M had high abundance of carbohydrate metabolism, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, and nucleotide metabolism among bacteria and archaea. The abundance of those functional genes categories respectively accounted for more than 27% and 30% of the bacteria and archaea. However, group U, L, and M had similar abundance in the same category and showed no significant difference among three groups.

### 3.3 Extracellular metabolites

The metabolites in group Cul were analyzed. The KEGG pathway and related metabolites were presented in Figure S2 and Table 3. A total number of 720 metabolites were detected, among which 311 metabolites were upregulated and 178 metabolites were downregulated. The FC of some metabolites was showed in Table S3.

Most metabolites were related to environmental information processing, metabolism, and/or organisal systems, whereas only a small part were related to drug development and/or genetic information processing. Some of metabolites are functional components such as gallic acid and quercetin. The metabolites could be divided into the following nine categories: (a) Carbohydrate, such as sucrose and stachyose. (b) Amino acid, such as L-phenylalanine, L-glutamic acid, and L-cystathionine. (c) Vitamin, such as vitamin A, pantothenic acid, and L-ascorbate. (d) Nucleotides, such as adenine, cytosine, and thymine. (e) Alkaloid, such as theophylline, betaine, and capsaicin. (f) Amines, such as phenethylamine, tyramine, and 3-methoxytyramine. (g) Acids, such as kinic acid, syringic acid, and ferulic acid. (h), Phenols, such as kaempferol, o-cresol, and xanthohumol. (i) Aldehydes, such as cuninaldehyde, 3,4-dihydroxybenzaldehyde, and phenylacetalddehyde.

### 3.4 Pearson correlation between the microbes and metabolites

Pearson correlation heat map showed the correlation between 10 microbes with the highest abundance and the 10 metabolites with highest abundance and five metabolites with lowest abundance (Figure 5).

* Methanobrevibacter, Methanolinea, Methanobacterium, Candidatus_Methanoplasm, Candida, and Lactarius showed significant positive correlation with Phenethylamine and trans-Aconitic acid. Methanobrevibacter, Lactarius, and Pseudopithomyces showed significant positive correlation with L-Phenylalanine. Alcaligenes, Ochrobactrum, Lactobacillus, Cladosporium, and Plectosphaerella showed significant positive correlation with D-Glucono-1, 5-lactone. Ralstonia, Methanomassiliicoccus, Malassezia, and Pseudopithomyces showed significant positive correlation with Styrene. Ralstonia and Methanomassiliicoccus showed significant positive correlation with Adenine. Haloarcula and Aspergillus showed significant positive correlation with Desbiobiotin. Methanobrevibacter and Lactarius showed significant positive correlation with Thymine. Methanoseta, Natronomonas, Halopenitus, Haloarcula, Methanospirillum, and Aspergillus showed significant positive correlation with Salicylic acid. Methyloptena, Methanoiline, Methanobacterium, Candidatus_Methanoplasm, Candida, and Lactarius showed significant positive correlation with trans-Cinnamic acid. Methanoseta, Natronomonas, Methanobrevibacter, and Fusarium showed significant positive correlation with Stachyose. Lactobacillus, Ralstonia, Methanoseta, Malassezia, and Pseudopithomyces showed significant positive correlation with Catharanthine. Haloarcula, Methanoseta, and Aspergillus showed significant positive correlation with L-Cystathionine. Alcaligenes, Ochrobactrum, Mortierella, and Plectosphaerella showed significant positive correlation with Scopolamine. In conclusion, Pearson analysis indicated that the metabolites can be contributed to these microbes.
### TABLE 3  Alpha diversity indices of different groups of bacteria, archaea, and fungi

| Group | OTU | Shannon | Simpson | ACE           | Chao 1          |
|-------|-----|---------|---------|---------------|-----------------|
| **Bacteria** | | | | | |
| U     | 1,630 | 6.04 ± 0.01 | 0.0082 ± 0.0005 | 1,306.64 ± 34.14 | 1,311.31 ± 31.40 |
| L     | 1,569 | 6.12 ± 0.04 | 0.0073 ± 0.0000 | 1,355.90 ± 58.51 | 1,364.20 ± 52.96 |
| M     | 1,550 | 6.03 ± 0.04 | 0.0086 ± 0.0000 | 1,318.27 ± 98.95 | 1,328.34 ± 93.62 |
| **Archaea** | | | | | |
| U     | 32   | 2.05 ± 0.08 | 0.1511 ± 0.0106 | 21.46 ± 3.85     | 18.83 ± 3.63    |
| L     | 28   | 2.11 ± 0.11 | 0.1557 ± 0.0155 | 26.38 ± 13.19    | 18.67 ± 1.20    |
| M     | 29   | 1.87 ± 0.20 | 0.2182 ± 0.0413 | 14.34 ± 7.24     | 18.67 ± 0.33    |
| **Fungi** | | | | | |
| U     | 417 | 1.26 ± 0.41 | 0.6266 ± 0.1105 | 191.67 ± 49.28   | 194.00 ± 51.56  |
| L     | 795 | 4.73 ± 0.04 | 0.0498 ± 0.0004 | 647.22 ± 14.64   | 647.21 ± 14.79  |
| M     | 475 | 2.28 ± 0.16 | 0.4462 ± 0.0349 | 228.17 ± 6.78    | 232.17 ± 9.29   |

**FIGURE 2** Composition of bacteria (a), archaea (b), and fungi (c) at the phylum level

**FIGURE 3** Composition of bacteria (a), archaea (b), and fungi (c) at the genus level
4 | DISCUSSION

In this study, the carbohydrate, protein, fat, total ash, and moisture content were 2.13%, 0.13%, 0.07%, 0.04%, and 88.3%, respectively, in the wet acTS. Proteobacteria, Euryarchaeota, and Ascomycota were the dominant phyla of bacteria, archaea, and fungi, respectively. Uncultured bacterium f_Anaerolineaceae, Alcaligenes, and Ochrobactrum were the three most abundant genera of bacteria; Methanoseta, Methanosphaera, and Natronomonas of archaea; Zygosacch- aromyces, Mortierella, and Fusarium of fungi. The contents of 311 metabolites increased, and the contents of 178 metabolites reduced in the acTS. Many of them were related to environmental information processing, metabolism, and organismal systems of KEGG pathway.

Our results showed that the acTS contains 2.13% carbohydrate, lower than that in ordinary food. The protein content of Taisui was significantly lower than the normal wild type of wet Taisui and also the standard of “high protein food” (12%) (Zhang Tao et al., 2018). In the meantime, the fat content was also significantly lower than that of the normal wet wild-type Taisui and the standard of “low fat food” (3%) (Chen et al., 2010). The concentration of total ash and trace elements was far lower than the normal concentration range of wet wild-type Taisui (Zhu et al., 2011). In short, the acTS used in current study is a substance with high protein. The differences between these two types of Taisui may due to their culture environment. Since all of carbohydrate, protein, fat, total ash, and trace elements were low, which means acTS is not a good source of macronutrients and trace elements.

In order to investigate whether or not it is safe to consume acTS as food ingredient, we have thus investigated the composition of microorganism in the acTS. In terms of bacteria, Proteobacteria contains Alcaligenes, Ochrobactrum, Methanosphaera, and Ralstonia, and Firmicutes contains Lactobacillus. These genus bacteria were dominant in this acTS as well. Alcaligenes, Methanolobacter, Ochrobactrum, and Ralstonia usually are pathogenic microorganism (Kalyuzhnaya et al., 2010; Li, 2018; Qi et al., 2019). Lactobacillus, the dominant bacteria of wild-type Taisui (Han et al., 2018), was also found. Lactobacillus is a well-known probiotic parasitizing in intestine and vagina (Setiarto et al., 2017). This acTS contains potential pathogenic bacteria and probiotics. However, it cannot infer that there is a safety risk in this acTS, because the safeness is not consistent for different bacterial strains. (Arelano et al., 2020).

In terms of archaea, Euryarchaeota contains many species including Methanoseta, Methanosphaera, Natronomonas, Methanoscircina, Methanobacterium, Methanospirillum, Methanobrevibacter, and Methanolina, which were the dominant genera of archaea in acTS. Previous report found that Methanobacterium, Methanobrevibacter, and Methanosphaera were the major part of wild-type Taisui (Wang...
Abundance of methanogenic archaea in patients with gastrointestinal and metabolic diseases was larger than normal people, which may be potentially detrimental for host health (Guo et al., 2015). However, it is still difficult to evaluate the safeness of consuming food with these archaea as the relationship between archaea and human health is not studied well.

For fungi, most of the dominant genera belong to Ascomycota, such as Zygosaccharomyces, Fusarium, Cladosporium, Candida, Penicillium, Chaetomium, Debaryomyces, Alternaria, Pichia, Aspergillus and Acremonium. Zygosaccharomyces, Dekkera, Debaryomyces, and Aspergillus glaucus are widely used in fermentation (Deng et al., 2009; Fang et al., 2006; Ishchuk et al., 2016; Zhang et al., 2016; Zhu et al., 2003). Dai (2007) reported that A. glaucus was the dominant fungi in wild Taisui. Lactarius, belonging to Chordata, can produce chemicals such as sesquiterpenes and has the activity of anti-tumor (Barros et al., 2007). Fortunately, these fungi are relatively safe because they are widely present in traditional foods. In addition, there are some commonly used industrial species in acTS, such as Mortierella for n-6 polyunsaturated fatty acids production, Pichia for protein, Chaetomium for cellulase, and Penicillium for penicillin (Ahmad et al., 2014; García-Estrada et al., 2020; Ho et al., 2007; Sun et al., 2019). The safeness of some genera of fungi in acTS is still undefined. Several types of Alternaria could cause diseases, but it could produce anticancer drugs such as vinblastine (Duan et al., 2008). Some species of Cladosporium can cause allergy (Bensch et al., 2012); Candida could cause inflammation (Tarang et al., 2020) and Malassezia could cause dandruff (Sommer et al., 2015). So, this acTS contains potential pathogenic and probiotics. However, it also cannot infer that the

**FIGURE 5** Pearson correlation between the metabolites and bacteria (a), archaea (b), and fungi (c)
acTS has a safety risk because different strains of fungi, even belonging to the same species, have different safety (Arellano et al., 2020). Due to the existence of potential unsafe microorganism in acTS, acTS metabolites were further investigated. The metabolites of acTS involve a large part of KEGG pathways, except for Cellular Processes and Human Diseases. Different metabolites have different functions. According to the function of the increased metabolites, they can be divided into the following five categories: (a) Improving...

| KEGG pathway | Number | Metabolites |
|--------------|--------|-------------|
| Nuclear receptors | 4 | Cortisone, Estradiol, Hydrocortisone, Estriol |
| Membrane transport | 12 | Octopine, Octopine, D-Mannitol, Carnitine, L-Phenylalanine, Choline, Inositol, Bettein, L-Ascorbate, D-Glucose-6-phosphate, Sucrose, Biotin |
| Signal transduction | 12 | Quercetin, Salicylic acid, Citric acid, D-Glucose 6-phosphate, L-Ascorbate, acetoacetate, Serotonin, Phosphoethanolamine, Adenosine, Jasmonic acid, Acetycholine, Inositol |
| Translation | 5 | L-Phenylalanine, L-Histidine, L-Tyrosine, L-Tryptophan, L-Glutamic acid |
| Amino acid metabolism | 51 | Octopine, Carnitine, Kynurenic acid, 6-Hydroxymethylalanin, Serotonin, L-Cystathionine, Urocanic Acid, Betaine, Creatinine, Phenylacetylglutamine, L-Phenylalanine, Indole, Tyramine, Picolinic acid, L-Dopa, L-Tryptophan, Sarcosine, L-Glutamic acid, acetoacetate, 4-Hydroxyphenylacetate, Citric Acid, Choline, Hydroquinone, Octopine, Phenethylamine, Citric acid, N-Acetyl-L-phenylalanine, 2-Isopropylmalic acid, Protocatechualdehyde, L-Hydroxyproline, N-Acetyl-D-phenylalanine, Capsaicin, Homoserine, Shikimic acid, Tyrosol, Homogentisic acid, Succiinic acid, 2,5-Dihydroxybenzaldehyde, Xanthurenic Acid, Phenol, trans-Cinnamic acid, Phenylacetaldehyde, Salicylic acid, 3-Methoxytyramine, L-Tyrosine, Fumaric Acid, Rosmarinic Acid, Piceolic acid, L-Serine, Choline, Acetylcholine, Tetrahydrocorticosterone, Adrenosterone, Hydrocortisone, Prostaglandin B2, 2-Methoxyestrone, Pyridoxamine 5-phosphate, trans-Tyrosine, L-Ascorbate, L-Histidine, L-Tyrosine, L-Tryptophan, L-Glutamic acid |

| Table 4 KEGG pathway and some related metabolites |
|-----------------------------|-----------------------------|
| KEGG pathway                | Number | Metabolites |
| Nuclear receptors           | 4      | Cortisone, Estradiol, Hydrocortisone, Estriol |
| Membrane transport          | 12     | Octopine, Octopine, D-Mannitol, Carnitine, L-Phenylalanine, Choline, Inositol, Bettein, L-Ascorbate, D-Glucose-6-phosphate, Sucrose, Biotin |
| Signal transduction         | 12     | Quercetin, Salicylic acid, Citric acid, D-Glucose 6-phosphate, L-Ascorbate, acetoacetate, Serotonin, Phosphoethanolamine, Adenosine, Jasmonic acid, Acetycholine, Inositol |
| Translation                 | 5      | L-Phenylalanine, L-Histidine, L-Tyrosine, L-Tryptophan, L-Glutamic acid |
| Amino acid metabolism       | 51     | Octopine, Carnitine, Kynurenic acid, 6-Hydroxymethylalanin, Serotonin, L-Cystathionine, Urocanic Acid, Betaine, Creatinine, Phenylacetylglutamine, L-Phenylalanine, Indole, Tyramine, Picolinic acid, L-Dopa, L-Tryptophan, Sarcosine, L-Glutamic acid, acetoacetate, 4-Hydroxyphenylacetate, Citric Acid, Choline, Hydroquinone, Octopine, Phenethylamine, Citric acid, N-Acetyl-L-phenylalanine, 2-Isopropylmalic acid, Protocatechualdehyde, L-Hydroxyproline, N-Acetyl-D-phenylalanine, Capsaicin, Homoserine, Shikimic acid, Tyrosol, Homogentisic acid, Succiinic acid, 2,5-Dihydroxybenzaldehyde, Xanthurenic Acid, Phenol, trans-Cinnamic acid, Phenylacetaldehyde, Salicylic acid, 3-Methoxytyramine, L-Tyrosine, Fumaric Acid, Rosmarinic Acid, Piceolic acid, L-Serine, Choline, Acetylcholine, Tetrahydrocorticosterone, Adrenosterone, Hydrocortisone, Prostaglandin B2, 2-Methoxyestrone, Pyridoxamine 5-phosphate, trans-Tyrosine, L-Ascorbate, L-Histidine, L-Tyrosine, L-Tryptophan, L-Glutamic acid |

- Biosynthesis of other secondary metabolites | 44     | L-Tryptophan, Kaempferol, Scopolamine, Caffeine, 7-Methylkynanthine, Scopoletin, Coniferin, Theophylline, Pinocembrin, 3,4-Dihydroxybenzaldehyde, Biochanin A, L-Phenylalanine, Indole, Tyramine, Picolinic acid, L-Dopa, Catharanthine, Colchicine, Xanthine, Galangin, L-Glutamic acid, Galangin, Papaverine, Inositol, Puromycin, Chrysin, Egonine, Piceatannol, Glycitein, Ferulic acid, Naringenin, Chalcone, Seneconine, Morphine, Quercetin, L-Tyrosine, Luteolin, Phenylpyruvic Acid, D-Glucose-6-phosphate, trans-Cinnamic acid, Xanthohumol, Chlorogenic acid, Formononetin, Naringenin, Piceolic acid, Genistein, 3-Methoxytyramine |

- Chemical structure transformation maps | 43     | Octopine, Kaempferol, Catharanthine, Caffeine, Scopolamine, Serotonin, 3,4-Dihydroxybenzaldehyde, Genistein, 3-Methoxytyramine, Theophylline, Ferulic acid, Tyramine, Biochanin A, Scopoletin, L-Phenylalanine, Colchicine, Piceolic acid, Papaverine, Xanthine, trans-Cinnamic acid, 4-Hydroxybenzaldehyde, L-Glutamic acid, L-Tryptophan, Jasmonic acid, Shikimic acid, L-Dopa, IMP, Piceolic acid, L-Tyrosine, Naringenin, Seneconine, Morphine, Mevalonic acid, Phenylpyruvic Acid, Fumaric Acid, Succinic acid, Capsaicin, Formononetin, Naringenin, Proteotocatechualdehyde, Citric acid, Gallic acid, 2,3-Dihydroxybenzoic acid, Salicylic acid, D-Glucose-6-phosphate, |

- Lipid metabolism | 20     | Estradiol, Thromboxane B2, Deoxycorticosterone, Jasmonic acid, Cortisone, Adrenosterone, Hydrocortisone, Prostaglandin B2, 2-Methoxyestrone, acetoacetate, Choline, Acetycholine, Tetradecyloctylcortosterone, Tetradecylocitcosterone, Lignoceric acid, Citric acid, Arachidic acid, estron3-sulfate, Eicosapentaenoic acid, Phosphoethanolamine |

- Metabolism of cofactors and vitamins | 17     | Vitamin A, 3-Succinoylpyridine, Uracil, 4-Pyridoxic acid, Dethiobiotin, L-Glutamic acid, Pyridoxine, Biotin, Pantothenic acid, Dihydrouracil, Porphobilinogen, L-Tyrosine, trans-Cinnamic acid, Homogentisic acid, Succinic acid, Fumaric Acid, Pyridoxamine 5-phosphate |

- Metabolism of terpenoids and polyketides | 9      | Adenine, Kanosamine, Eucalyptol, Carvone, Mevalonic acid, L-Tyrosine, Gibberellic acid, Perillic acid, Salicylic acid |

- Digestive system | 22     | Vitamin A, Thromboxane B2, Octopine, Paracetamol, Pantothenic acid, L-Phenylalanine, Choline, Serotonin, Hydrocortisone, Indole, Acetycholine, Biotin, Tyramine, L-Tryptophan, Phenol, 4-Methylphenol, Sucrose, Salicylic acid, 4-Methylphenol, Salicylic acid, Pyridoxamine, 5-phosphate, D-Glucose 6-phosphate, estrone 3-sulfate, L-Ascorbate, Phenol, Sucrose, estrone 3-sulfate, Pyridoxamine, 5-phosphate, D-Glucose 6-phosphate, L-Ascorbate |
color, fragrance, and taste of food, such as L-glutamic acid, vanillyl alcohol, and cuminaldehyde; (b) Preventing the damage of spoilage bacteria and pathogenic bacteria, such as protocatechucic acid, quercetin, and luteolin. Luteolin have the antibacterial ability, and the higher the concentration, the more the antibacterial; (c) Enhancing nutrition and maintaining healthy, such as ferulic acid, desthiobiotin, and deoxyadenosine. The herbs that full of ferulic acid is used for curing thrombosis in China for a long time (Ou & Kwok, 2004). Desthiobiotin could have the influence of gene expression like biotin (Rodríguez-Melendez et al., 2003); (d) Anticancer, anti-inflammatory; anti-diseases, anti-oxidation, such as kaempferol, chlorogenic acid, ferulic acid, genistein, luteolin, naringenin, gallic acid, protocatechucic acid, quercetin, L-ascorbate, genistein, luteolin, and cytidine. Genistein could inhibit the carcinogenesis in animal models (Banerjee et al., 2008). Quercetin has excellent antioxidant capacity in vitro within the flavonoid family (Boots et al., 2008); (e) Chemical and pharmaceutical intermediates or product, such as salicylic acid, terephthalic acid, styrene, thymine, cytosine, hypoxanthine, uracil, 4-hydroxybenzal-dehyde, α-cresol, 4-hydroxyphenylacetic acid, and 3,4-di-hydroxybenzaldehyde. 4-hydroxybenzaldehyde is artificially synthesized into drug with anti-inflammatory activities (Lim et al., 2008). Salicylic acid is pharmaceutical intermediates, which is used for anti-inflammatory medicine like aspirin (Su et al., 2017). In conclusion, most of the metabolites is beneficial to human.

The KEGG pathways of functional genes prediction and extracellular metabolites were consistent. Pearson correlation analysis showed that some microbes are significantly associated with the above metabolites. According to previous report, Aspergillus is one of the most important strains commercially produced citric acid by starch or sucrose-based medium fermentation (Aboyeji et al., 2020). It is known that honey contains a high amount of sucrose and thus is a good source for Aspergillus to produce citric acid. As reported, Candida could also be potentially used to produce citric acid (Uzah et al., 2020). Aspergillus produces D-biotin with a structure similar that of desthiobiotin, suggesting that Aspergillus may potentially produce desthiobiotin (Zheng, 2007). Candida can utilize its own lipase to catalyze phenylthethylamine synthesis from lipid substrate (Wen, 2012). Except for Candida, the main organisms commonly used to produce stereoselective lipases are Pseudomonas, Fusarium, and Aspergillus (Qin, 2006). Therefore, microbes may also be responsible for the production of metabolites. This study indicates that the acTS used in current study could be potentially developed into fermented food, functional food, or oral liquid.

5 | CONCLUSION

In current study, the chemical compositions, the microbial structure of bacteria, archaea, and fungi, and the extracellular metabolites of acTS were analyzed. The concentrations of macronutrient and trace elements were low, indicating that acTS will not be a good source of macronutrients and trace elements. Microbial composition of bacteria, archaea, and fungi, and metabolomics analysis showed that acTS can provide some probiotics and some beneficial metabolites for human. It is therefore possible to develop acTS into fermented food, functional food or oral liquid, only if when safeness of each microorganism is proved.

ACKNOWLEDGMENTS

The program was supported by the funds of Key-Area Research and Development Program of Guangdong Province (Nos. 2020B020226005 and 2020B020226008). The authors thank Ruixia Qiu, Bing Yu and Shulin Deng from the Department of Food Science and Engineering, Jinan University, for their contributions to this study. We would also like to thank Biomarker Technologies Co., Ltd., (Beijing, China) for the analysis of Microbial Community Diversity, and Novogene Co., Ltd., (Beijing, China) for the analysis of Non-targeted Metabolomics.

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

ORCID

Guangwen Zhang https://orcid.org/0000-0003-2257-151X
Junsheng Liu https://orcid.org/0000-0003-2481-4415
Xichun Peng https://orcid.org/0000-0002-4804-5203

REFERENCES

Aboyeji, O. O., Oloke, J. K., Arinkoola, A. O., Oke, M. A., & Ishola, M. M. (2020). Optimization of media components and fermentation conditions for citric acid production from sweet potato peel starch hydrolysate by Aspergillus niger. Scientific African, 10, e00554. https://doi.org/10.1016/j.sciaf.2020.e00554
Ahmad, M., Hirz, M., Pichler, H., & Schwab, H. (2014). Protein expression in Pichia pastoris: Recent achievements and perspectives for heterologous protein production. Applied Microbiology and Biotechnology, 98(12), 5301–5317. https://doi.org/10.1007/s00253-014-5732-5
Albalasmeh, A. A., Berhe, A. A., & Ghezzehei, T. A. (2013). A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydrate Polymers, 97(2), 253–261. https://doi.org/10.1016/j.carbpol.2013.04.072
Arellano, K., Vazquez, J., Park, H., Lim, J., Yi, Y., Kang, H.-J., Cho, D., Jeong, H. W., & Holzapfel, W. H. (2020). Safety evaluation and whole-genome annotation of Lactobacillus plantarum strains from different sources with special focus on isolates from green tea. Probiotics Antiinocb Proteins. 12, 1057–1070. https://doi.org/10.1007/s12602-019-09620-y
Banerjee, S., Li, Y., Wang, Z., & Sarkar, F. H. (2008). Multi-targeted therapy of cancer by genistein. Cancer Letters, 269(2), 226–242. https://doi.org/10.1016/j.canlet.2008.03.052
Barros, L., Baptista, P., & Ferreira, I. C. F. R. (2007). Effect of Lactarius piperus fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food Chemical Toxicology, 45(9), 1731–1737. https://doi.org/10.1016/j.fct.2007.03.006
Bensch, K., Braun, U., Groenewald, J. Z., & Crous, P. W. (2012). The genus Cladosporium. Studies in Mycology, 72, 1–401. https://doi.org/10.3114/sim0003
Bitter, N. Q., Fernandez, D. P., Driscoll, A. W., Howa, J. D., & Ehleringer, J. R. (2020). Distinguishing the region-of-origin of roasted coffee beans with trace element ratios. Food Chemistry, 320, 126602. https://doi.org/10.1016/j.foodchem.2020.126602
Boots, A. W., Haenen, G. R. M. M., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology*, 585, 325–337. https://doi.org/10.1016/j.ejphar.2008.03.008

Chen, H., Wang, D., Li, X., & Wang, X. (2010). Orthogonal-array-design optimization of extraction technologies of polysaccharide from soybeans. *Food Science*, 31(4), 6–10.

Chiachung, C. (2003). Evaluation of air oven moisture content determination methods for rough rice. * Biosystems Engineering*, 86(4), 447–457. https://doi.org/10.1016/jbiosystemseng.2003.08.010

Dai, L. (2007). Assessment of mycoxymycete and fungal diversity of the “mycoxymyccete complex”. (M.Eng.), Northwestern University, Xian.

Deng, M., Wu, X., Li, Q., Huang, L., Bao, S., & Long, M. (2009). Purification and properties of α-glucosidase from Aspergillus glaucus EU7-22. *Development and Application*, 19(6), 61–63. https://doi.org/10.16519/j.cnki.2011.335

Duan, L., Chen, H., Chen, J., Li, W., & Hong, L. (2008). Screening the high-yield pacitaxel producing strain Alternaria alternata var. monosporus. *Chinese Journal of Antibiotics*, 11(3), 650–653.

Dunn, W. B., Broadhurst, D., Begley, P., Zelena, E., Francis-McIntyre, J., & Edgar, R. C. (2013). Uparse: Highly accurate OTU sequences from microbial communities. *Nature Methods*, 10(10), 996–998. https://doi.org/10.1038/nmeth.2604

Fang, H., Cao, Y., Lu, J., & Xie, G. (2006). The molecular identification & classification of the main mold from wheat starter. *Liquor-making Science & Technology*, 3, 45–47.

García-Estrada, C., Martín, J. F., Cueto, L., & Barreiro, C. (2020). Omics approaches applied to *Penicillium chrysogenum* and penicillin production: Revealing the secrets of improved productivity. *Genes*, 11(6), 712. https://doi.org/10.3390genes11060712

Geiser, D. M., del Mar Jiménez-Gasco, M., Kang, S., Makalowska, I., Veeraraghavan, N., Ward, T. J., Zhang, N., Kuldau, G. A., & O’donnell, V. S., Anderson, N., Brown, M., Knowles, J. D., Halsall, A., Haselden, J. G. (2011). Identification and characterisation of the main mold from wheat starter. *Letters in Biotechnology*, 42(2), 400–410.

Han, X., Zhang, X., Yan, Y., Zheng, Y., Feng, H., & Zhao, T. (2018). Bacterial diversity of the Yellow River TaiSai by 16S rRNA gene sequencing. *Microbiology China*, 45(4), 866–874. https://doi.org/10.13344/j.microbiol.china.170406

Ho, S., Jiang, Y., & Chen, F. (2007). Polysaturated fatty acids (PUFAs) content of the fungus *Mortierella alpina* isolated from soil. *Journal of Agricultural & Food Chemistry*, 55(10), 3960–3966.

Ishchuk, O. P., Vojvoda Zeljko, T., Schifferdecker, A. J., Mebrahtu Wisen, S., Hagström, Å. K., Rozpedowska, E., Rerdam Andersen, M., Hellborg, L., Ling, Z., Sibiry, A., & Pliskur, J. (2016). Novel centromeric loci of the wine and beer yeast *Dekkera bruxellensis* CEN1 and CEN2. *PLoS One*, 11(8), e0161741. https://doi.org/10.1371/journal.pone.0161741

Kalyuzhnaya, M. G., Beck, D. A., Suci, D., Pozhitkov, A., Lidstrom, M. E., & Chistoserdova, L. (2010). Functioning in situ: Gene expression in *Methyloptera mobilis* in its native environment as assessed through transcriptomics. *International Society for Microbial Ecology*, 43(3), 388–398. https://doi.org/10.1016/ismej.2009.117

Lakshanomya, N., Danudol, A., & Ningnoi, T. (2011). Method performance study for total solids and total fat in coconut milk and products. *Journal of Food Composition and Analysis*, 24(4), 650–655. https://doi.org/10.1016/j.jfca.2010.10.002

Li, E., Ren, J., Chen, Q., Zhang, H., Lin, B., Zhang, H., Zhang, S., Xiang, C., & Li, L. (2020). Uncovering the mysterious identity of TaiSai—An old Chinese folk legend. *Science China Life Science*, 63(12), 1942–1945. https://doi.org/10.1007/s11427-019-1640-6

Li, H., Huang, J., Feng, J., Su, X., Wnag, W., & Yang, M. (2015). The research progress of TaiSai. *Farm Products Processing*, 6, 73–77. https://doi.org/10.3969/j.issn.1671-9646(X).2015.06.023

Li, Z. (2018). Symbiotic bacteria of pregnancy and infants in perinatal period and their dependency. (Ph.D.), Jinan University, Guangzhou.

Lim, E., Kang, H., Jung, H., Kim, K., Lim, C., & Park, E. (2008). Anti-inflammatory, anti-angiogenic and anti-nociceptive activities of 4-hydroxybenzaldehyde. *Biomolecules & Therapeutics*, 16(3), 231–236. https://doi.org/10.4062/biomolther.2008.16.3.231

Lin, J., Xiong, X., Ge, X., Wang, J., Su, X., & Zhang, W. (2013). Isolation and identification of microbial strains in two different TaiSai samples. *Letters in Biotechnology*, 24(6), 825–827. https://doi.org/10.3969/j.issn.1000-0002.2013.06.017

Oellig, C., Link, K., & Schwack, W. (2020). Characterization of E 472 food emulsifiers – Determination of bound and free fruit acids, free glycerol and ash content. *Journal of Chromatography A*, 1619, 460946. https://doi.org/10.1016/j.jchroma.2020.460946

Oftedal, O. T., Eisert, R., & Barrell, G. K. (2014). Comparison of analytical and predictive methods for water, protein, fat, sugar, and gross energy in marine mammal milk. *Journal of Dairy Science*, 97(8), 4713–4732. https://doi.org/10.3168/jds.2014-7895

Ou, S., & Kwo, K. (2004). Ferulic acid: Pharmaceutical functions, preparation and applications in foods. *Journal of the Science of Food and Agriculture*, 84(11), 1261–1269. https://doi.org/10.1002/jsfa.1873

Qi, Y., Li, S., Qu, D., Chen, L., Gong, R., Gao, K., & Sun, Y. (2019). Effects of ginseng neutral polysaccharide on gut microbiota in antibiotic-associated diarrhea mice. *China Journal of Chinese Material Medica*, 44(4), 811–818.

Qin, S. (2006). Chiral resolution of racemates by immobilized lipase. (M.Eng.), Beijing University of Chemical Technology, China.

Rodriguez-Melendez, R., Lewis, B., McMahon, R. J., & Zempleni, J. (2003). Diaminobiotin and desthiobiotin have biotin-like activities in Jurkat cells. *Nutrient-Genes Interactions*, 133(5), 1259–1264. https://doi.org/10.1093/jn/133.5.1259

Setiarto, R. H. B., Widhyastuti, N., Saskiawan, I., & Safitri, R. M. (2017). The inulin variation concentration effect in fermentation using *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. *Biopropal Industri*, 8(1), 1–17.

Sommer, B., Overy, D. P., & Kerr, R. G. (2015). Identification and characterization of lipases from *Malassezia restricta*, a causative agent of dandruff. *FEMS Yeast Research*, 15(7), fov078. https://doi.org/10.1093/femsyr/fov078

Su, D., Wang, W., & Chen, Y. (2017). Analysis of contents of free salicylic acid in aspirin enteric-coated tablets from different manufacturers. *Tianjin Pharmacy*, 29(4), 8–10.

Sui, T. (2019). Studies on the induction of apoptosis and autophagy colon cancer HCT116 by extract of Meat-like Ganoendrma. (M.Eng.), Jilin University, Changchun.

Sun, D., Wen, A., Li, X., Lin, Z., Xiao, C., & Zhu, L. (2019). Cellulase production by mixed fungi solid fermentation and the application in soybean straw degradation. *Soysbean Science*, 38(1), 49–55.

Svenja, H., Kevin, Q., Charmion, C. Q., Leping, L., Rick, R., Nichole, R., & Manisha, P. (2016). Exploratory metabolomics profiling in the kaenic acid rat model reveals depletion of 25-hydroxyvitamin D3 during epileptogenesis. *Scientific Reports*, 6, 31424.

Tarang, S., Keshwerani, V., Latendresse, B., Lindgren, L., Sanchez, S. M. R., & Weston, M. D. (2020). In silico design of a multivitamin vaccine
against Candida albicans. Scientific Reports, 10(1066), 1–7. https://doi.org/10.1038/s41598-020-57906-x

Tong, J., Xiong, X., Wang, J., Xu, W., & Zhang, W. (2018). Preliminary study of strains isolated from different “TaiSui” samples. Letters in Biotechnology, 29(2), 238–242. https://doi.org/10.3969/j.issn.1009-0002.2018.02.016

Uzah, G. A., Akani, N. P., & Odu, N. N. (2020). Screening of Aspergillus and Candida species with utmost potential to synthesize citric acid. Journal of Advances in Microbiology, 20(4), 10–18. https://doi.org/10.9734/jamb/2020/v20i42023

Wang, C., & Wang, S. (2017). Analysis of bacterial diversity and community structure from three Taisui based on high-throughput sequencing. Hubei Agricultural Sciences, 56(13), 2543–2547. https://doi.org/10.14088/j.cnki.issn0439-8114.2017.13.037

Wang, C., & Wang, S. (2014). Statistical analysis confirms “Taisui” is a kind of indigenous objective object. Acta Agriculturae Boreali Sinica, 5, 199.

Wang, C., & Wang, S. (2017). Study of archaea community structure on different forms of Taisui. Biotechnology, 27(3), 276–281. https://doi.org/10.16519/j.cnki.1004-311x.2017.03.0045

Wang, P. (2018). Study on anti-tumor activity of Ganoderma lucidum extract. (M.Eng.), Changchun University of Science and Technology, Changchun.

Wang, X. (2007). Preliminary research on bacterial diversity and inhibition function of “large myxomycete-like complex”. (M.Eng.), Northwest University, Xian.

Wen, S. (2012). High-level heterologous expression and in vitro molecular evolution of lipase Lip2 from Yarrowia lipolytica, and its application in kinetic resolution. (M.Eng.), Beijing University of Chemical Technology, Beijing.

Zhang, J., Fang, F., Chen, J., & Du, G. (2016). Metabolism of ethyl carbamate precursors in soy sauce by Zygosaccharomyces rouxii ZQ02. Acta Microbiologica Sinica, 56(6), 956–963. https://doi.org/10.13343/j.cnki.wsxb.20150394

Zhang Tao, G. J., Xiaoquan, Y., Manyuan, H. E., Zixu, W., & Shuyan, H. (2018). Stability of high protein food system constructed with soy protein particles. Modern Food Science and Technology, 34(11), 57–63. https://doi.org/10.13982/j.mfst.1673-9078.2018.11.010

Zheng, J. (2007). Microbial enzymatic preparation of chiral biotin intermediate lactine in organic solvent. (M.Eng.), Zhejiang University of Technology, Hangzhou.

Zhu, C., Bai, T., Jiang, Q., Zheng, F., Ai, H., Zhu, J., & Liu, H. (2011). Biological components of “Tai Sui”. Journal of Microbiology, 31(1), 1–5.

Zhu, Y., Zhao, G., & Shuai, G. (2003). A mixed culture with Debaryomyces vanriji and Saccharomyces cerevisiae and the influence of it on the flavor of wine. Liquor-making Science & Technology, 4, 70–72.

SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Chen Y, Zheng S, Zhang G, Luo J, Liu J, Peng X. Chemical, microbial, and metabolic analysis of Taisui cultured in honey solution. Food Sci Nutr. 2021;9:2158–2168. https://doi.org/10.1002/fsn3.2185