Evaluating the effect of lactic acid bacterial fermentation on salted soy whey for development of a potential novel soy sauce-like condiment

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A B S T R A C T

There were two main objectives of this study: (1) to understand the effect of salt concentration on the growth of four lactic acid bacteria (LAB) in soy whey and determine the non-volatile and volatile profiles generated after fermentation; (2) to evaluate the potential of using salted soy whey to develop a sauce-like condiment through LAB fermentation. The four LAB included non-halophilic *Lactiplantibacillus plantarum* ML Prime, *Limosilactobacillus fermentum* PCC, *Oenococcus oeni* Enoferm Beta and halophilic *Tetragenococcus halophilus* DSM20337. At 2% salt, all LAB grew remarkably from day 0 to day 1, except for *T. halophilus*, while at 6% salt, the growth of *L. plantarum*, *L. fermentum* and *O. oeni* was suppressed. Conversely, the higher salt concentration enhanced the growth of *T. halophilus* in soy whey as the cell count only increased from 6.36 to 6.60 log CFU/mL at 2% salt but it elevated from 6.61 to 7.55 log CFU/mL at 6% salt. Similarly, the higher salt content negatively affected the sugar and amino acids metabolism and organic acids production by non-halophilic LAB. *L. plantarum* and *O. oeni* generated significantly ($p <$ 0.05) more lactic acid (3.83 g/L and 4.17 g/L, respectively) than *L. fermentum* and *T. halophilus* (2.02 g/L and 0 g/L, respectively) at 2% salt. In contrast, a higher amount of acetic acid was generated by *L. fermentum* (0.72 g/L at 2% salt) and *T. halophilus* (0.51 g/L at 6% salt). LAB could remove the green and beany off-flavours in soy whey by metabolizing C6 and C7 aldehydes. However, to develop a novel soy sauce-like condiment, yeast fermentation and Maillard reaction may be required to generate more characteristic soy sauce-associated aroma compounds.

1. Introduction

Tofu, or soybean curd, is a popular soybean product that is widely consumed around the world, especially in Asian countries. Tofu can be produced by adding a coagulant to freshly boiled soymilk, followed by pressing the precipitated protein to remove excess water (soy or tofu whey) (Chua and Liu, 2019). Soy whey, a highly perishable liquid by-product, is generated in large volumes from tofu processing. It has been reported that for every 1 kg of soybeans used to manufacture tofu, up to 8.98 kg of soy whey can be generated (Fei et al., 2017). Most of the soy whey is discarded as waste effluent by tofu manufacturers due to the lack of economically viable technology and the desire to utilize it (Chua and Liu, 2019). However, disposing of soy whey without treatment not only pollutes the environment, but is also a waste of resources as soy whey still contains a fair amount of nutrients. In recent years, many attempts have been made to reuse soy whey through nutrient recovery or biotransformation. For example, soy whey has been used as a substrate to develop soy alcoholic beverages (Chua et al., 2017), soy sauce (Xu et al., 2021) and probiotic functional beverages (Tu et al., 2019). These biotransformation technologies of regular soy whey prove that

soy whey can serve as a good substrate for the growth of microorganisms.

Salted pressed tofu (*tau kwa*) is a popular type of soybean curd in Southeast Asia, particularly in Singapore. Salted pressed tofu production is very similar to regular tofu production, except that salt is added to the soymilk before coagulation and an extra amount of water is pressed out to make the tofu texture firmer. Therefore, a greater amount of soy whey is produced from *tau kwa* production. More importantly, the addition of salt poses an additional challenge to soy whey valorisation. Therefore, a zero-waste approach to effectively adding value to salted soy whey is necessary.

As one of the most consumed seasonings/condiments in East and Southeast Asian cuisines, soy sauce has also been gaining popularity in Western countries (Diez-Simon et al., 2020). This dark brown liquid with an intense umami, salty and caramel-like flavour is usually prepared by fermenting soybeans in brine. In traditional soy sauce production, a complex microbial community of fungi, yeasts and bacteria is formed in a non-sterile environment and thus, fermentation control is difficult. Although soy sauce production process varies in different countries, koji culturing (solid-state fermentation) and moromi (or

mash) fermentation are two commonly shared steps. At the beginning of moromi fermentation, lactic acid bacteria (LAB) grow very rapidly, reducing the pH of the mash to around 4.0 to 5.0 to impart a sour taste to soy sauce and to provide an environment suitable for the growth of soy sauce yeasts (Devanthi and Gkatzionis, 2019). Yeasts play significant roles in the generation of aroma compounds in soy sauce. In modern soy sauce production, microorganisms are often inoculated as starter cultures to achieve consistent soy sauce product quality.

Tetragenococcus halophilus is a halophilic bacterium that has been reported to be the most predominant LAB in soy sauce fermentation (Devanthi and Gkatzionis, 2019). In brine, it acts as a starter culture to generate lactic acid and acetic acid, resulting in moromi acidification. In addition, *T.halophilus* was found to improve the overall volatile compounds profiles of fermented fish sauce as it produced numerous aroma compounds, including 2-methylpropanal, 2-methylbutanal, 3-methylbutanal and benzaldehyde during fermentation (Udomsil et al., 2011). Other than *T. halophilus*, many other non-halophilic LAB were indicated to be important in flavour modulation through fermentation and these LAB have potential but have not been explored in developing low-salt sauce-like fermented products.

Recently we explored the feasibility of transforming salted soy whey into a soy sauce-like condiment using wine yeast *Torulaspora delbrueckii* and soy sauce yeasts *Zygosaccharomyces rouxii* and *Candida versatilis* as single starter cultures (Zhou et al., 2022). In the current study, four LAB including *Lactiplantibacillus plantarum* ML Prime, *Limosilactobacillus fermentum* PCC, *Oenococcus oeni* Enoferm Beta and *T. halophilus* DSM20337 were selected as single starter cultures for soy sauce-like condiment fermentation to understand their effect on flavour changes of soy whey. The objectives of this study were (1) to determine the growth of four LAB in soy whey with 2% and 6% salt and to investigate the flavour profiles of salted soy whey after fermentation; (2) to evaluate the feasibility of using LAB to transform salted soy whey into a reduced-salt soy sauce-like condiment.

2. Materials and methods

2.1. Bacterial cultures preparation

Four LAB were used in this study. *L. plantarum* ML Prime and *L. fermentum* PCC were obtained from Lallemand Inc. (Montreal, Canada) and Chr. Hansen (Hoersholm, Denmark), respectively. *O. oeni* Enoferm Beta and *T. halophilus* DSM20337 were purchased from Lallemand Inc. (Edwardstown, Australia) and DSMZ (Braunschweig, Germany), respectively. Freeze-dried cultures of LAB were activated and cultured before use. *L. plantarum* ML Prime and *L. fermentum* PCC were transferred individually to sterile De Man, Rogosa and Sharpe (MRS) broth (Oxoid Ltd., Hampshire, United Kingdom). Freeze-dried *O. oeni* Enoferm beta was activated in MRS broth containing 20% (v/v) apple juice and 80% (v/v) MRS broth and *T. halophilus* DSM20337 was propagated in sterile tryptic soya broth (TSB) containing 6% (w/v) salt. The inoculated broths were incubated statically at 30 °C for 3 days to obtain pure cultures and stored at −80 °C before use.

2.2. Soy whey collection and media preparation

The generation of soy whey was carried out as described by Chua et al. (2018). Briefly, soybeans (from Canada) were soaked in deionized water at a ratio of 1:6 (w/v) for 16 h. Soaked soybeans were drained and blended with deionized water (1:6 dry weight to volume) to obtain a slurry. The slurry was then pressed and filtered through a cheesecloth, separating okara (soybean pulp) from soymilk. The okara was rinsed in a 1:2 dry weight to volume ratio with deionized water. The soymilk obtained was boiled for 5 min and then cooled to 87 °C before adding calcium sulphate to coagulate. Once the soymilk temperature reached 87 °C, a commercial calcium sulphate water slurry (10% w/v) was added to soymilk at 2% (w/v with respect to the dry weight of the

soybeans) dosage. The soymilk mixture was then stirred continuously for 1 min before letting it stand for 15 min for coagulation. The coagulated tofu was pressed to collect soy whey.

Commercial glucose was added to the soy whey to adjust the soluble solids content (°Brix) to 8 (initial °Brix value ranging from 1.80 to 2.00). The soluble solids content was measured using a refractometer (RX-5000α, ATAGO, Tokyo, Japan). After that, soy whey was divided into two treatment groups, one with 2% (w/v) salt added and the other with 6% (w/v) salt added. The soluble solids level and salt contents adjusted soy whey were then pasteurized at 105 °C for 30 min.

2.3. Fermentation design

The frozen pure cultures of LAB were transferred to respective sterilized nutrient broth (MRS broth for *L. plantarum* ML Prime and *L. fermentum* PCC; MRS broth containing 20% (v/v) apple juice for *O. oeni* Enoferm Beta; TSB consisting of 6% (w/v) salt for *T. halophilus* DSM20337) individually and incubated at 30 °C for 24 h. The LAB pre-cultures were prepared by inoculating the reactivated LAB into pasteurized soy whey containing 2% salt at a ratio of 5% (v/v). The pre-cultures were then incubated at 30 °C for another 24 h before actual fermentation.

During actual fermentation, 3 mL of each pre-culture was added into 300 mL of pasteurized soy whey containing 2% and 6% salt, respectively. The whole experiment was performed in duplicate and repeated once (total 4 replicates per treatment). The inoculated salted soy whey was incubated at 30 °C for 7 days under static conditions. Samples were withdrawn periodically for viable cell count, pH measurement (Mettler Toledo, Giessen, Germany) and subsequent chemical analysis.

2.4. Microbial enumeration

To determine the viability and cell count of LAB in salted soy whey, 1 mL of samples from each replicate was diluted serially with 1 g/L bacteriological peptone and enumerated on MRS agar (*L. plantarum*, *L. fermentum* and *O. oeni*) and tryptic soya agar (TSA) (*T. halophilus*) using the spread plate method. These MRS and TSA plates were incubated at 30 °C for 4 days prior to colony counting.

2.5. Non-volatile compounds analysis

Sugars (glucose, fructose and sucrose) and organic acids contents were analysed using high-performance liquid chromatography (HPLC; Shimadzu, Kyoto, Japan). The sample preparation, detection, and quantification methods were identical to that of our previous study (Zhou et al., 2022). Briefly, prior to analysis, samples were centrifuged at $11,000 \times g$ for 10 min and filtered through 0.20-μm microfilters. For sugars, isocratic elution using a mobile phase comprising of acetonitrile/water/triethylamine (80:19.8:0.2 v/v/v) at 40 °C, with a 1.0 mL/min flow rate was performed through an XBridge Amide column (150 × 4.6 mm, Waters, Milford, Massachusetts, United States). Samples were detected with an evaporative light scattering detector (ELSD).

For organic acid analysis, a Supelcogel C–610H column (300 × 7.8 mm, Supelco, Bellefonte, PA, United State) and 0.1% (v/v) sulphuric acid (0.4 mL/min, 40 °C) were used as the stationary and mobile phase, respectively. Free amino acids were analysed by using a pre-set physiological separation program on ARACUS Amino Acid Analyzer (MembraPure, Berlin, Germany).

2.6. Volatile compounds analysis

Volatile compounds analysis was carried out using the method described by Chua et al. (2017). Briefly, unfermented and fermented salted soy whey samples were adjusted to pH 2.5 using 1 M HCl. After pH adjustment, 5 mL of each sample was transferred to a 20 mL-vial individually. The volatile compounds were extracted using the headspace

solid-phase microextraction (HS-SPME) method with a carboxen/polydimethylsiloxane fibre (85 μm; Supelco). Gas chromatography (GC; Agilent 6890A) coupled with a mass spectrometer (MS; Agilent 5975C) and flame ionization detector (FID; Agilent Technologies) were used to analyse and detect the volatiles. Volatiles were characterized and identified by comparing their mass spectra (MS) with the Wiley 275 MS libraries and the National Institute of Standards and Technology (NIST 14) library and later verified based on linear retention index (LRI) values. Butyl butyryl lactate (0.05 ppm in methanol) (Mane SEA Pte Ltd., Singapore) was added into each sample as an internal standard.

2.7. Statistical analysis

All data reported were obtained from four independent replicates (n = 4) and expressed as the mean ± SD. Two-way ANOVA ($p < 0.05$) was conducted to understand the relationship of two factors (salt level and bacterial species) of the fermented soy whey. The Windows SPSS 20.0 software (SPSS Corporation, Chicago, IL, USA) was used to perform two-way ANOVA, one-way ANOVA, and Tukey's post-hoc test. Principal component analysis (PCA) was carried out by SIMCA Version 13.0 (Umetrics, Umeå, Sweden) to identify the difference between volatile compounds among unfermented and fermented samples.

Fig. 1. Changes of (a) growth kinetics and (b) pH of four LAB during 7-day fermentation in salted soy whey (●) *L.. plantarum* (▲) *L.; fermentum* (✕); *O. oeni* (■); *T. halophilus*. Dashed lines represent LAB in soy whey with 2% salt while solid lines represent that of 6% salt.

3. Results and discussion

3.1. LAB growth and pH changes

The growth kinetics of LAB in salt-added soy whey are shown in Fig. 1a. In soy whey with 2% salt, all LAB grew and exhibited evident growth except for *T. halophilus*. *L. plantarum*, *L. fermentum* and *O. oeni* shared a very similar log phase growth trend in soy whey with 2% salt and the cell counts of the three LAB increased remarkably from 7.19 to 8.86 log CFU/mL (*L. plantarum*), from 6.61 to 8.69 log CFU/mL (*L. fermentum*) and from 7.23 to 8.87 log CFU/mL (*O. oeni*) in 1 day and declined thereafter. The cell count of *L. fermentum* at 2% salt experienced a more significant decrease from day 2 to day 5 compared to the other two LAB. As for *T. halophilus* at 2% salt, although the cell number increased significantly ($p < 0.05$) by day 7, the growth was greatly impeded. In contrast, *T. halophilus* grew well at 6% salt. Therefore, soy whey with 6% salt was more optimal for the growth of *T. halophilus* as compared to 2%. This result was expected because *T. halophilus* has been reported to be a moderately halophilic LAB which is strictly chloride dependent and 3%–17.5% NaCl in media is preferred by *T. halophilus* (Liu et al., 2015). At 5% salt, the growth of *T. halophilus* was enhanced (Justé et al., 2012). In our study, the lower cell count of *T. halophilus* at day 0 could be attributed to the fact that the growth of *T. halophilus* was significantly inhibited in pre-culture which the LAB was inoculated into soy whey containing 2% salt.

Suppressed growth of non-halophilic *L. plantarum*, *L. fermentum* and *O. oeni* at 6% salt was observed (Fig. 1a). The higher osmotic stress reduced the growth and survival of the three LAB by decreasing positive turgor pressure (Mbye et al., 2020). Water migrates out of the bacterial cells leading to dehydration. To counter such changes, *L. plantarum*, *L. fermentum* and *O. oeni* must adjust their intracellular osmolality to maintain the turgor pressure. Different salt stress adaptation mechanisms have been reported for different bacteria to survive and grow with accumulating compatible solutes such as glycine betaine and proline as one of the most common strategies (De Angelis and Gobbetti, 2004; Zhao et al., 2014).

As illustrated in Fig. 1b, the pH of all fermentation setups decreased significantly ($p < 0.05$) by the end of fermentation. Compared to other LAB, *T. halophilus* fermented samples had the least pH reduction. Although the pH of *T. halophilus* at 2% salt experienced a significant ($p < 0.05$) decrease, the pH drop was minimum (from pH 5.54 to 5.34). This could be ascribed to the least cell biomass production at 2% salt (Fig. 1a). At 6% salt, the pH of *T. halophilus* fermented samples dropped to 4.88 on day 3 and remained unchanged until the end, agreeing with Devanthi et al. (2018) that the pH of moromi inoculated with pure *T. halophilus* decreased to 4.83 by the end of fermentation. The optimal pH for *T. halophilus* is around 7.0 and once the pH drops below 5.0, the bacterium is no longer able to grow.

L. plantarum and *O. oeni* had very similar pH reduction trends (Fig. 1b). In soy whey with 2% salt, the pH of *L. plantarum* and *O. oeni* dropped from 5.36 and 5.32 to 3.40 and 3.35, respectively. These two bacteria had the lowest pH among the four LAB which coincided with the highest lactic acid production (Fig. 3a). Salt had a negative impact on the growth of *L. plantarum* and *O. oeni*, and thus negatively affected the pH reduction rate. Compared to *L. plantarum* and *O. oeni,* the pH reduction of *L. fermentum* was restricted more significantly. The pH of *L. fermentum* fermented soy whey dropped from 5.45 to 3.95 (2% NaCl) and 4.56 (6% NaCl). Li and Liu (2021) also reported that when inoculating *L. fermentum* into unsalted pork hydrolysates, pH decreased from 6 to around 4.5 by the end of fermentation. At both 2% and 6% salt, *L. fermentum* had a lesser pH drop compared to *L. plantarum* and *O. oeni* (Fig. 1b). This could be caused by the lesser lactic acid produced by *L. fermentum* (Fig. 3a). Finished soy sauce has a pH of around 4.7 (Luh, 1995), thus *L. plantarum* and *O. oeni* fermented samples had undesirable low pH, affecting the flavour of the condiment. Only higher salt (6% NaCl) soy whey inoculated with *L. fermentum* (pH of 4.56) and
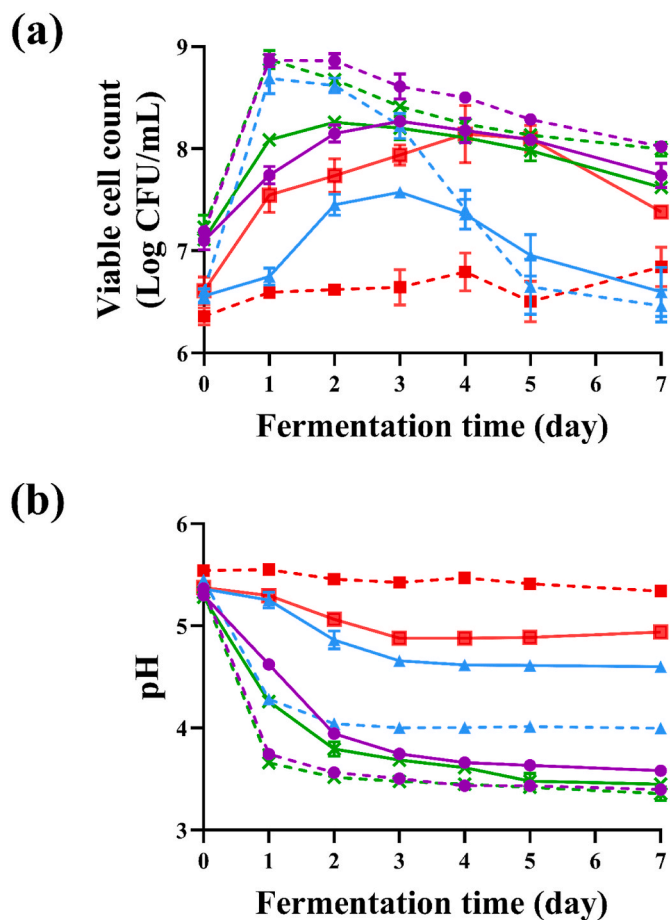
T. halophilus (pH of 4.88) had final pH close to the regular commercial soy sauce pH of 4.7.

3.2. Changes in sugars and organic acids during fermentation

Changes in sugars (glucose, fructose and sucrose) in LAB fermented salted soy whey are shown in Fig. 2. In soy whey with 2% salt, all LAB except for *T. halophilus* were able to metabolize glucose significantly ($p < 0.05$), reducing glucose from 69.6 g/L to around 45.0 g/L (Fig. 2a). Comparatively, at 6% salt, none of the four LAB utilized glucose. A similar observation in sauerkraut fermentation was reported by Yang et al. (2019), with higher salt concentrations resulting in higher amounts of residual glucose and fructose. Salt concentration had a significant impact on the glucose metabolism of *L. plantarum*, *L. fermentum* and *O. oeni*, with a high salt concentration exhibiting an inhibitory effect. There was no significant ($p > 0.05$) decrease in glucose observed in soy whey (both 2% and 6% NaCl) inoculated with *T. halophilus*. The capability of glucose metabolism in *T. halophilus* could be strain-dependent as some studies indicated that *T. halophilus* lack the ability to utilize glucose while others had the opposite conclusion (Chun et al., 2019; Devanthi et al., 2018; Justé et al., 2008; Roling and van Verseveld, 1996).

As shown in Fig. 2b, fructose concentration of all fermentation setups except for *T. halophilus* at 2% salt decreased significantly ($p < 0.05$) after fermentation. Compared to glucose consumption of *L. plantarum*, *L. fermentum* and *O. oeni*, fructose metabolism was less affected by the additional amount of salt in soy whey. As for *T. halophilus*, higher salt concentration (6%) promoted the utilization of fructose. This correlated with the higher cell biomass in soy whey with 6% salt (Fig. 1a). *T. halophilus* preferred fructose than glucose in soy whey with 6% salt. Many studies concluded that *T. halophilus* harboured metabolic pathways for fructose (Chun et al., 2019; Justé et al., 2008), but there is no study conducted to ascertain if *T. halophilus* is fructophilic LAB. At 6% salt, none of the four LAB utilized sucrose while *L. plantarum* and *L. fermentum* consumed sucrose at 2% salt (Fig. 2c). Sucrose content of *L. plantarum* and *L. fermentum* fermented salted soy whey with 2% salt decreased from 3.13 g/L to 2.51 g/L (*L. plantarum*) and 2.55 g/L (*L. fermentum*). Vrancken et al. (2009) also reported that at a higher salt

concentration (6%), less sucrose was depleted by *L. fermentum* than that at 2% salt. However, the exact mechanism is still not well understood.

Organic acid contents in salted soy whey before and after fermentation are shown in Fig. 3. Lactic acid plays an important role in soy sauce flavour as lactic acid imparts a sour taste to soy sauce while making saltiness milder (Singracha et al., 2017). Kong et al. (2018) reported that lactic acid, ranging from 0.83 to 13.19 g/L, is a dominant organic acid in commercial soy sauce. As expected, significant amounts of lactic acid ($p < 0.05$) were generated by all LAB, except for *T. halophilus* at 2% NaCl (Fig. 3a). *T. halophilus* has been reported as an obligate homofermenter that performs homolactic fermentation to generate only lactic acid from glucose (Justé et al., 2012). However, a recent study suggested that *T. halophilus* possesses the ability to carry out homolactic and heterolactic fermentation, utilizing various carbohydrate sources to produce lactic acid, acetic acid and ethanol (Chun et al., 2019, 2021). At 2% salt, *T. halophilus* did not generate lactic acid. This could be due to its limited growth under undesirable condition. In soy whey with 6% salt, around 0.65 g/L of lactic acid was generated by *T. halophilus*. A similar observation was noted by Devanthi et al. (2018), whereby fermenting *T. halophilus* in moromi containing 10% salt increased lactic acid content to around 0.6 g/L.

L. plantarum and *O. oeni* fermented samples had higher concentrations of lactic acid produced at both 2% and 6% NaCl, which was in line with their higher cell counts and greater pH reduction (Fig. 1). Salt had a negative effect on lactic acid production of *L. plantarum*, *L. fermentum* and *O. oeni*, as more lactic acid was produced at lower salt levels. Other studies also concluded higher salt concentrations impeded lactic acid production by LAB (Xiong et al., 2016; Yang et al., 2019). The generation of lactic acid in LAB mainly originated from sugar metabolism. Sugars were firstly converted to pyruvic acid and then reduced to lactic acid (Gao et al., 2020). Other than sugar metabolism, lactic acid could also be generated from citric acid (Gao et al., 2020; Lonvaud-Funel, 1999), and some amino acids such as aspartic acid and serine (Table 1) degradation (Wakinaka et al., 2019). For example, some LAB including *L. fermentum* possess serine dehydratase activity which utilizes serine and preferentially converts it to pyruvate (Fernández and Zúñiga, 2006). The pyruvate produced could further be transformed into lactate, acetate or acetolactate.
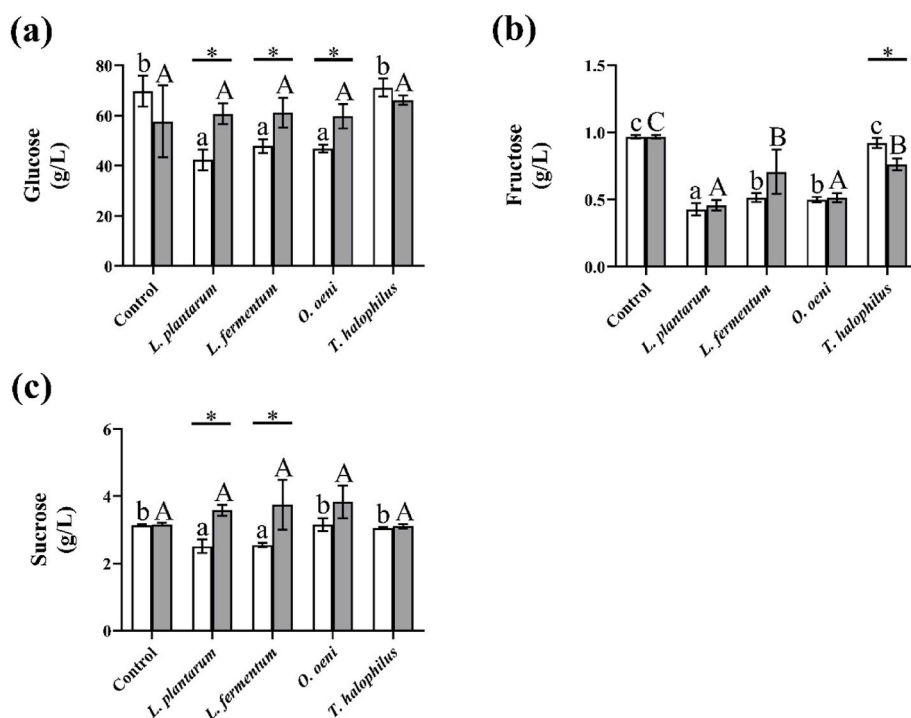

(a)

(b)

(c)

Fig. 2. Initial and final sugar concentrations ((a) glucose (b) fructose and (c) sucrose) in unfermented and fermented soy whey with 2% (represented by unshaded bars) and 6% salt (represented by shaded bars).
[a, b, c]: Different lower-case letters indicate significant differences ($p < 0.05$) for unfermented and fermented soy whey with 2% salt.
[A, B, C]: Different upper-case letters indicate significant differences ($p < 0.05$) for unfermented and fermented soy whey with 6% salt. *Asterisks indicate significant differences ($p < 0.05$) between 2% and 6% salt for each LAB.
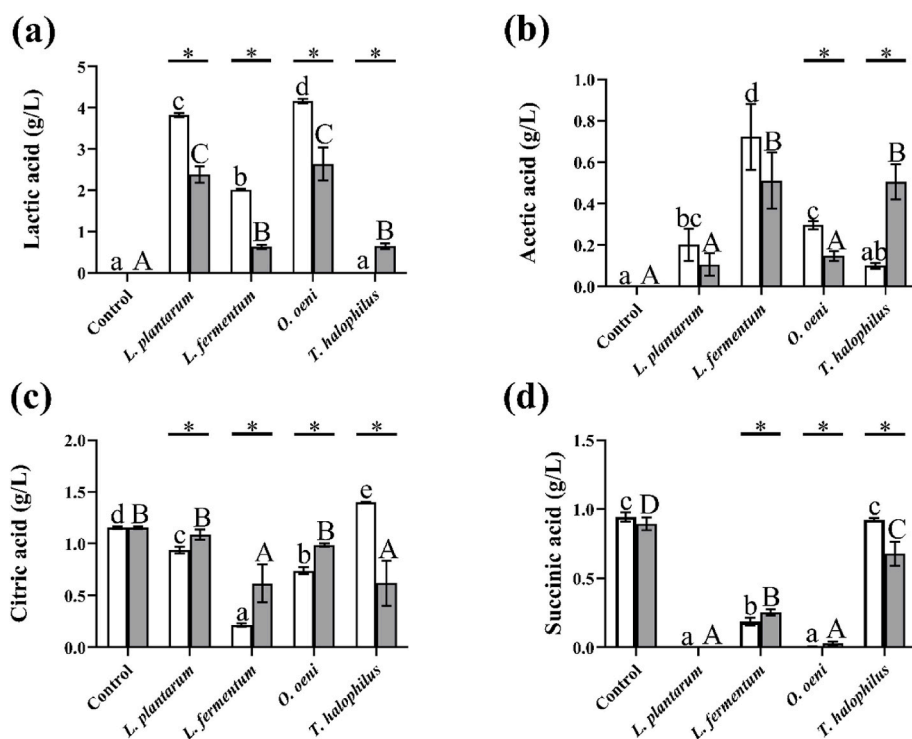
Fig. 3. Initial and final organic acid concentrations ((a) lactic acid, (b) acetic acid, (c) citric acid and (d) succinic acid) in unfermented and fermented soy whey with 2% (represented by unshaded bars) and 6% salt (represented by shaded bars).

[a, b, c, d, e]: Different lower-case letters indicate significant differences ($p < 0.05$) for unfermented and fermented soy whey with 2% salt.

[A, B, C, D]: Different upper-case letters indicate significant differences ($p < 0.05$) for unfermented and fermented soy whey with 6% salt. *Asterisks indicate significant differences ($p < 0.05$) between 2% and 6% salt for each LAB.

Acetic acid is another important organic acid that is usually found in soy sauce; it imparts a sour odour and taste to soy sauce while neutralizing the saltiness, making the soy sauce more refreshing (Liu et al., 2021). As shown in Fig. 3b, there were considerable amounts of acetic acid produced by all LAB during soy whey fermentation. Among them, *L. fermentum* generated the highest level of acetic acid (0.72 g/L) at 2% salt. *L. fermentum* and *O. oeni* are obligate heterofermentative LAB which could catabolize sugars through the phosphoketolase (PPK) pathway to generate lactic acid, ethanol/acetic acid, and carbon dioxide. Compared to *O. oeni*, *L. fermentum* produced significantly less lactic acid but more acetic acid. Additionally, after fermentation, citric acid in *L. fermentum* samples was significantly lower than that in other LAB fermented samples. This implies that the higher level of acetic acid in *L. fermentum* could be generated from citric acid catabolism. In our study, a relatively high amount of acetic acid was found in *T. halophilus* at 6% salt. Under aerobic condition, acetic acid was reported to be the main product of *T. halophilus* metabolism (Gürtler et al., 1998). As discussed above, *T. halophilus* harboured a facultative lactic acid fermentation pathway which generates acetic acid during fermentation (Chun et al., 2019). Also, it is suggested that *T. halophilus* converts citric acid to acetic acid and ethanol via pyruvate formate lyase (Gänzle, 2015).

A decreasing trend of citric acid was observed in all LAB fermentations at 2% salt except for *T. halophilus* (Fig. 3c). At 6% salt, only *T. halophilus* and *L. fermentum* samples had citric acid contents decreased from 1.16 g/L to 0.62 g/L, metabolizing around 48% of citric acid. The decreased levels of citric acid might contribute to the production of acetic acid and oxaloacetate by citrate lyase during LAB fermentation (Bekal et al., 1998). In addition, in some *Lactobacillus* species, citric acid could be converted to succinic acid via the reductive pathway of the tricarboxylic acid (TCA) cycle (Dudley and Steele, 2005). Surprisingly, the citric acid content in *T. halophilus* fermentation at 2% NaCl was found to be elevated significantly after fermentation ($p < 0.05$). It may be speculated that the increased citric acid content could contribute to the synthesis of proline (an osmoprotectant), which requires a continuous supply of precursor α-oxoglutarate and glutamate (He et al., 2017a). Hahne et al. (2010) also indicated that when *Bacillus subtilis* was under osmotic stress, the enzymes leading to the synthesis of

α-oxoglutarate remained present while the enzymes responsible for converting oxoglutarate to oxaloacetate were reduced. In this way, bacteria make sure an adequate supply of oxoglutarate can be converted into glutamate and further synthesize proline. The high proline concentration in *T. halophilus* fermented soy whey (Table 1) is in accordance with the speculation. Contrary to the well-known production of succinic acid by LAB from citric acid, we found succinic acid (Fig. 3d) declined after LAB fermentation, except for *T. halophilus* at 2% NaCl. A similar result was reported by (Gao et al., 2020). It is possible that LAB consumed succinic acid as a substrate to generate other metabolites. However, the exact mechanism is still unknown.

3.3. Changes in free amino acids contents during fermentation

Amino acids not only play a crucial role in LAB growth, but also are important taste-active compounds and aroma precursors. Changes in free amino acids before and after LAB fermentation are shown in Table 1. In general, all LAB fermented samples experienced a decrease in total amino acid contents, of which *L. fermentum* fermented salted soy whey at 2% NaCl had the least residual amount of total amino acids after fermentation, decreasing from 301.81 mg/L to 84.50 mg/L. In contrast, *T. halophilus* had the highest residual total amino acid content after fermentation.

Salt negatively affected the amino acids metabolism of *L. plantarum*, *L. fermentum* and *O. oeni*. Comparatively, the additional salt elevated the total amino acids consumption of *T. halophilus* (consumed 16.24 mg/L at 2% salt, 34.59 mg/L at 6% salt), supporting the halophilic nature of *T. halophilus*. Higher osmotic pressure results in less consumption of proline in all LAB fermentations or more production, which is in line with the result reported by He et al. (2017a). Proline, a universal compatible solute, is often accumulated in many LAB to combat high osmotic stress (He et al., 2017a). With a higher level of proline in LAB, increased amounts of intermediates involved in glycolysis, the TCA cycles and the pentose phosphate pathway were observed in bacterial cells (He et al., 2017b). Glycine is the precursor of glycine betaine which is another universal and effective compatible osmoprotectant that LAB and plants can accumulate in response to salt stress (He et al., 2017c).

Table 1
Change of free amino acid contents in unfermented and LAB fermented soy whey.

| | Day 0 | Day 7 | | | | | | | | Test of significance between effects, $P^{\&}$ | | |
| | | 2% | | | | 6% | | | | Main effect | | Interaction |
	Untreated soy whey[a]	L. plantarum	L. fermentum	O. oeni	T. halophilus	L. plantarum	L. fermentum	O. oeni	T. halophilus	Salt	Bacteria	Salt x Bacteria
Amino acids (mg/L)												
Aspartic acid	9.35 ± 0.18	0.28 ± 0.04ᵃ	0.80 ± 0.05ᵃᵇ	8.89 ± 1.63ᵉ	7.13 ± 0.33ᵈ	0.32 ± 0.02ᵃ	0.23 ± 0.08ᵃ	1.94 ± 0.67ᵇ	3.87 ± 0.32ᶜ	Sig.*	Sig.	Sig.
Threonine	8.04 ± 0.11	0.66 ± 0.10ᵃ	0.58 ± 0.21ᵃ	0.67 ± 0.20ᵃ	10.63 ± 0.27ᶜ	2.29 ± 0.27ᵇ	0.00 ± 0.00ᵃ	2.02 ± 0.36ᵇ	13.94 ± 1.00ᵈ	Sig.	Sig.	Sig.
Serine	6.64 ± 0.10	0.30 ± 0.12ᵃᵇ	0.37 ± 0.08ᵃᵇ	0.88 ± 0.33ᵇ	8.01 ± 0.09ᵉ	1.71 ± 0.16ᶜ	0.00 ± 0.00ᵃ	2.68 ± 0.11ᵈ	8.35 ± 0.57ᵉ	Sig.	Sig.	Sig.
Asparagine	27.32 ± 0.39	9.46 ± 1.16ᵇ	0.00 ± 0.00ᵃ	11.29 ± 1.51ᵇᶜ	23.98 ± 1.28ᵉ	14.51 ± 0.22ᶜᵈ	13.56 ± 1.16ᶜ	17.32 ± 1.37ᵈ	26.66 ± 2.78ᵉ	Sig.	Sig.	Sig.
Glutamic acid	11.51 ± 0.50	6.83 ± 1.27ᵃ	8.77 ± 2.59ᵃ	27.12 ± 6.62ᵇᶜ	14.01 ± 0.68ᵃ	11.89 ± 0.86ᵃ	29.66 ± 0.46ᶜ	21.97 ± 4.86ᵇ	6.91 ± 0.94ᵃ	Sig.	Sig.	Sig.
Glycine	6.96 ± 0.23	0.85 ± 0.16ᵇ	0.49 ± 0.08ᵃᵇ	1.95 ± 0.10ᶜ	7.27 ± 0.25ᵉ	2.30 ± 0.13ᶜ	0.00 ± 0.00ᵃ	3.69 ± 0.60ᵈ	8.03 ± 0.51ᶠ	Sig.	Sig.	Sig.
Alanine	15.36 ± 0.28	3.42 ± 0.42ᵃᵇ	21.36 ± 1.46ᶠ	2.40 ± 0.43ᵃ	16.34 ± 0.43ᵉ	5.19 ± 0.88ᵇᶜ	12.05 ± 2.08ᵈ	6.85 ± 0.38ᶜ	17.47 ± 1.54ᵉ	N. S.	Sig.	Sig.
Valine	23.84 ± 0.68	26.52 ± 0.45ᵇ	20.14 ± 1.87ᵃ	23.64 ± 3.64ᵃᵇ	26.72 ± 1.36ᵇ	23.39 ± 2.35ᵃᵇ	23.07 ± 0.52ᵃᵇ	31.95 ± 1.60ᶜ	25.64 ± 2.13ᵇ	Sig.	Sig.	Sig.
Cystine	4.07 ± 0.28	2.75 ± 0.15ᵃ	2.92 ± 0.55ᵃᵇ	3.00 ± 0.32ᵃᵇ	3.76 ± 0.13ᶜ	2.71 ± 0.36ᵃ	2.88 ± 0.15ᵃᵇ	3.18 ± 0.17ᵃᵇᶜ	3.58 ± 0.40ᵇᶜ	N. S.	Sig.	N. S.
Methionine	6.23 ± 0.43	1.14 ± 0.20ᵇ	0.00 ± 0.00ᵃ	0.62 ± 0.17ᵇ	3.70 ± 0.08ᵉ	2.71 ± 0.37ᶜᵈ	2.48 ± 0.15ᶜᵈ	2.90 ± 0.33ᵈ	2.34 ± 0.33ᶜ	Sig.	Sig.	Sig.
Isoleucine	7.80 ± 0.18	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	0.28 ± 0.03ᵃ	7.66 ± 0.12ᵉ	2.83 ± 0.27ᶜ	1.00 ± 0.06ᵇ	1.60 ± 0.38ᵇ	5.06 ± 0.65ᵈ	Sig.	Sig.	Sig.
Leucine	13.57 ± 0.29	1.36 ± 0.21ᵃ	0.86 ± 0.20ᵃ	1.11 ± 0.13ᵃ	13.83 ± 0.26ᵈ	6.29 ± 0.55ᵇ	6.49 ± 0.28ᵇ	6.39 ± 0.95ᵇ	11.66 ± 0.93ᶜ	Sig.	Sig.	Sig.
Tyrosine	18.37 ± 0.55	0.63 ± 0.10ᵃ	2.29 ± 0.65ᵇ	0.00 ± 0.00ᵃ	17.85 ± 0.21ᵉ	6.58 ± 0.24ᶜ	9.70 ± 0.23ᵈ	5.77 ± 0.94ᶜ	17.44 ± 1.29ᵉ	Sig.	Sig.	Sig.
Phenylalanine	17.26 ± 0.48	1.48 ± 0.19ᵃ	3.20 ± 1.16ᵇ	0.98 ± 0.20ᵃ	16.29 ± 0.22ᵉ	7.12 ± 0.62ᶜ	9.97 ± 0.28ᵈ	7.00 ± 0.96ᶜ	15.57 ± 0.89ᵉ	Sig.	Sig.	Sig.
Histidine	4.13 ± 0.23	3.73 ± 0.29ᵃ	3.10 ± 0.35ᵃ	3.84 ± 1.09ᵃ	4.03 ± 0.16ᵃ	2.88 ± 0.12ᵃ	3.25 ± 0.29a	3.48 ± 0.60ᵃ	4.01 ± 0.48ᵃ	N. S.	Sig.	N. S.
Tryptophan	17.34 ± 1.32	8.24 ± 0.67ᶜᵈ	8.39 ± 0.84ᶜᵈ	4.61 ± 1.19ᵃ	7.39 ± 0.60ᵇᶜ	8.07 ± 0.44ᶜᵈ	9.36 ± 1.10ᵈ	5.95 ± 0.61ᵃᵇ	7.52 ± 0.58ᵇᶜᵈ	N. S.	Sig.	N. S.
Lysine	19.95 ± 0.60	12.50 ± 0.46ᵃᵇ	11.22 ± 0.80ᵃ	14.89 ± 2.26ᵇᶜ	19.93 ± 0.52ᵈ	12.73 ± 0.80ᵃᵇ	16.28 ± 1.09ᶜ	14.69 ± 1.21ᵇᶜ	19.12 ± 1.07ᵈ	Sig.	Sig.	Sig.
Arginine	74.30 ± 2.34	69.43 ± 2.04ᵈ	0.00 ± 0.00ᵃ	60.95 ± 8.25ᶜᵈ	65.20 ± 1.67ᶜᵈ	50.41 ± 2.65ᵇ	0.00 ± 0.00ᵃ	67.50 ± 4.99ᵈ	57.46 ± 3.57ᵇᶜ	Sig.	Sig.	Sig.
Proline	9.76 ± 0.54	0.00 ± 0.00ᵃ	0.00 ± 0.00ᵃ	2.55 ± 0.59ᵇ	11.84 ± 0.19ᵈ	2.84 ± 0.20ᵇ	0.00 ± 0.00ᵃ	5.18 ± 0.56ᶜ	12.58 ± 0.82ᵈ	Sig.	Sig.	Sig.
Total amino acids	301.81 ± 6.92	149.57 ± 2.97ᵇ	84.50 ± 7.62ᵃ	169.70 ± 23.76ᵇ	285.57 ± 4.23ᵈ	166.76 ± 4.71ᵇ	140.00 ± 4.77ᵇ	212.09 ± 18.79ᶜ	267.22 ± 16.30ᵈ	Sig.	Sig.	Sig.
GABA (mg/L)	52.42 ± 2.17	53.77 ± 2.19ᶜ	44.38 ± 6.30ᵇ	42.46 ± 4.71ᵇ	46.85 ± 0.89ᵇᶜ	32.78 ± 1.15ᵃ	41.65 ± 2.98ᵇ	40.80 ± 2.92ᵃᵇ	48.30 ± 2.91ᵇᶜ	Sig.	Sig.	Sig.

$^{\&}$Two-way ANOVA was conducted for day 7 samples. Post-hoc Tukey's test was carried out to determine if there were any significant differences observed in the 2-way ANOVA analysis. Mean values in the same row with different superscript lower-case letters are significantly different ($p < 0.05$).

*Sig.: Significant difference ($p < 0.05$); N. S.: Non-significant differences ($p > 0.05$).

[a] Untreated soy whey: soy whey without glucose and salt supplementation and LAB fermentation.

However, *O. oeni* was concluded to lack the sequences homologous to the genes coding for proline and/or glycine betaine transporter, thus being unable to relieve salt stress (Le Marrec et al., 2007). Instead, accumulation of aspartate was considered to be protective for *O. oeni* cells when encountering high salt conditions. Aspartate could have been resulted from asparagine deamination via asparaginase, being consistent with lower levels of asparagine after fermentation except *T. halophilus* (Table 1). Surprisingly, there was more aspartic acid found in *O. oeni* fermented soy whey at 2% salt than at 6% salt which could be due to its higher cell biomass and asparaginase at 2% salt. Aspartic acid may protect the cell either through restoration of cell turgor or by serving as a substrate for metabolic pathways or biosynthesis which can help the cell combat dehydration (Le Marrec et al., 2007). For example, like other LAB, *O. oeni* could potentially decarboxylate aspartic acid to form alanine, creating a proton-motive force that is sufficiently high to drive ATP synthesis via the F0F1 ATPase (which is required under osmotic conditions) (Le Marrec et al., 2007). However, it is still unsure if *O. oeni* has the capability to convert aspartic acid to alanine like many other LAB.

Aspartate and glutamate are two important amino acids that contribute to the umami taste in soy sauce (Diez-Simon et al., 2020). *O. oeni* has the highest amount of free aspartate after fermentation in soy whey with 2% NaCl, followed by *T. halophilus*. As for key umami-imparting free glutamic acid, the highest concentration was found in *L. fermentum* fermented soy whey with 6% salt. Nonetheless, the glutamic acid content in fermented soy whey (Table 1) was much lower than in regular soy sauce (ranging from 2.5 to 11.8 g/L). Apart from umami taste, some amino acids have also been associated with bitterness. For example, arginine has an extremely bitter and unpleasant taste (Diez-Simon et al., 2020). Arginine was completely utilized by *L. fermentum* in salted soy whey fermentation, likely through the arginine deiminase (ADI) pathway (Vrancken et al., 2009). Compared to *L. fermentum*, other LAB fermented soy whey samples had significantly ($p < 0.05$) higher amounts of arginine, possibly due to weaker ADI activities. A similar result was reported by Li et al. (2021) that the concentration of arginine in pork trimming hydrolysate decreased significantly after being fermented with *L. fermentum* PCC. The complete utilization of arginine by *L. fermentum* is considered to be desirable as it can reduce the bitter taste in fermented soy whey.

Cysteine/cystine and methionine are important sulphur-containing amino acids which contribute to the soy sauce flavour development (Wong et al., 2008). The concentration of cystine and methionine decreased after LAB fermentation (Table 1). In fermented soy whey, these two amino acids only accounted for around 1.9% and 1.0% of the total amino acids determined, respectively. At the same time, there were no sulphur-containing volatile compounds detected in fermented soy

Table 2

Selected volatile compounds and their concentration (μg/L) in 2% salted soy whey before and after lactic acid bacterial fermentation.

Compound identification	Identification methods	LRI[a]		Day 0	Day 7			
		Ref	Expt	Unfermented soy whey	*L. plantarum*	*L. fermentum*	*O. oeni*	*T. halophilus*
Acids								
Acetic acid	MS, LRI	1451[b]	1420	65.22 ± 13.36a	81.47 ± 21.63a	301.56 ± 40.35b	101.42 ± 19.07a	60.07 ± 4.06a
Hexanoic acid	MS, LRI	1822[c]	1801	146.51 ± 21.41b	140.91 ± 26.65 b	106.50 ± 17.54 ab	93.40 ± 20.10a	106.39 ± 15.83 ab
Octanoic acid	MS, LRI	2038[c]	2010	13.24 ± 3.38 ab	15.50 ± 3.49 ab	12.87 ± 1.42a	18.79 ± 2.51b	12.58 ± 1.60a
Nonanoic acid	MS, LRI	2110[d]	2115	8.05 ± 1.19a	7.99 ± 2.03a	9.18 ± 1.71a	9.05 ± 0.95a	9.61 ± 0.52a
			Subtotal	233.02 ± 24.93	245.87 ± 44.89	430.11 ± 58.87	222.66 ± 36.00	188.65 ± 17.67
Alcohols								
Ethanol	MS			0.00 ± 0.00a	870.14 ± 194.44cd	1138.56 ± 204.75d	534.86 ± 81.37b	610.08 ± 38.66bc
1-Pentanol	MS, LRI	1248[e]	1231	44.70 ± 0.94b	50.83 ± 8.25b	24.50 ± 3.21a	24.20 ± 3.89a	20.59 ± 1.05a
1-Hexanol	MS, LRI	1350[e]	1330	107.53 ± 3.71a	596.35 ± 99.09c	346.25 ± 31.78b	300.93 ± 31.06b	171.31 ± 5.88a
1-Octen-3-ol	MS, LRI	1456[c]	1419	217.25 ± 20.20c	144.50 ± 29.06b	32.66 ± 9.20a	60.64 ± 14.33a	64.54 ± 3.90a
			Subtotal	369.47 ± 24.42	1661.81 ± 277.26	1541.97 ± 234.42	920.62 ± 103.61	866.52 ± 44.61
Aldehydes								
Hexanal	MS, LRI	1078[b]	1057	371.43 ± 51.19b	3.98 ± 1.19a	2.44 ± 0.45a	1.74 ± 0.42a	4.41 ± 0.07a
2-Hexenal	MS, LRI	1221[c]	1197	36.23 ± 6.77c	2.34 ± 0.99a	0.27 ± 0.05a	1.50 ± 0.51a	16.80 ± 0.75b
2-Heptenal	MS, LRI	1324[c]	1298	25.58 ± 2.42c	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	9.92 ± 0.55b
2-Octenal	MS, LRI	1429[c]	1401	11.63 ± 1.82b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Furfural	MS, LRI	1452[e]	1447	8.67 ± 0.92c	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	5.29 ± 0.36b
Benzaldehyde	MS, LRI	1530[c]	1506	4.80 ± 0.85b	10.16 ± 0.99d	2.01 ± 0.38a	7.50 ± 0.37c	3.99 ± 0.20b
			Subtotal	458.34 ± 60.49	16.48 ± 2.81	4.71 ± 0.80	10.75 ± 0.96	36.22 ± 8.53
Ketones								
β-Damascenone	MS, LRI	1857[f]	1785	20.98 ± 1.75b	29.52 ± 2.80c	23.72 ± 3.55b	20.75 ± 2.56b	13.74 ± 1.05a
			Subtotal	20.98 ± 1.75	29.52 ± 2.80	23.72 ± 3.55	20.75 ± 2.56	13.74 ± 1.05
Volatile phenols								
4-Ethylphenol	MS, LRI	2142[f]	2136	0.00 ± 0.00a	9.91 ± 1.67b	0.00 ± 0.00a	9.49 ± 1.36b	0.00 ± 0.00a
			Subtotal	0.00 ± 0.00	9.91 ± 1.67	0.00 ± 0.00	9.49 ± 1.36	0.00 ± 0.00
			Total	1081.82 ± 101.01	1963.58 ± 314.21	2000.51 ± 282.98	1184.27 ± 134.22	1105.13 ± 63.65

a, b, c, d: Statistical analysis using one-way ANOVA at 95% confidence interval (n = 4) and Tukey's post-hoc test. Mean values in the same row with different lower-case letters are significantly different ($p < 0.05$).

Concentration was determined using butyl butyryl lactate as an internal standard.

[a] LRI: Linear retention index.

[b] Li et al. (2021).

[c] Chua et al. (2018).

[d] Comuzzo et al. (2006).

[e] Ohata et al. (2017).

[f] López et al. (1999).

whey (Table 2 and Table 3), implying that LAB were unable to utilize sulphur-containing amino acids to produce volatile sulphur-containing compounds or the levels were too low to be detected.

3.4. Changes in volatile components during fermentation

The changes of different selected volatiles before and after fermentation are shown in Tables 2 and 3. The most abundant volatile group identified in unfermented soy whey belonged to aldehydes, which is consistent with the result reported by Chua et al. (2020). Of all, hexanal, which originated from the enzymatic and oxidative breakdown of linoleic acid, was the most predominant volatile in unfermented soy whey (371.43 μg/L at 2% NaCl and 405.94 μg/L at 6% NaCl).

In general, most aldehydes were metabolized to low levels after LAB fermentation while new aroma compounds, especially corresponding alcohols and acids were formed. Most of the aldehydes found in soybean products impart a green and beany odour with low-detection thresholds (Vong and Liu, 2018). Thus, the significant decrease ($p < 0.05$) of aldehydes is a desirable feature of LAB fermentation. LAB likely possess the ability to convert aldehydes to alcohols and carboxylic acids through enzymatic reduction and enzymatic oxidation of aldehydes, respectively (Li et al., 2021). For example, hexanal was mainly reduced to 1-hexanol by all LAB. The least amounts of aldehydes were detected in *L. fermentum* fermented soy whey, indicating that *L. fermentum* exhibited the greatest ability in aldehyde reduction (Gao et al., 2020), possibly because of its

heterofermentative nature and higher alcohol dehydrogenase activity. Correspondingly, at 2% NaCl, *L. fermentum* produced the highest amount of ethanol of 1138.56 μg/L.

Acids belong to the second most abundant volatile group in fermented soy whey. *L. fermentum* (at 2% and 6% NaCl) and *T. halophilus* (at 6% NaCl) produced the highest amount of acetic acid than other LAB under the same salt condition. This finding is in line with the content of HPLC-determined acetic acid discussed above. For other volatile acids, there was no obvious change after fermentation. In this study, *L. plantarum* and *O. oeni* were found to be the only two LAB that were able to produce 4-ethylphenol that may contribute a smoky/spicy flavour to fermented soy whey at both 2% and 6% salt. Some LAB can generate volatile phenols from phenolic acids via decarboxylation. The conversion involves the sequential activity of two enzymes: cinnamate decarboxylase decarboxylates *p*-coumaric acid into 4-vinylphenol; a reductase then converts 4-vinylphenol to 4-ethylphenol (Silva et al., 2011).

Compared to regular soy sauce, many characteristic flavour compounds (e.g. esters, furanones, phenols and pyrazines) are still missing in LAB-fermented salted soy whey. For example, 2,5-dimethyl-4-hydroxy-3 (2H)-furanone (4-HDMF) and tautomers 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone (4-HEMF) are reported to be key flavour compounds in soy sauce that contribute to caramel-like odour. The formation of 4-HDMF and 4-HEMF has been associated with Maillard reaction (Diez-Simon et al., 2020). Additionally, esters including ethyl

Table 3

Selected volatile compounds and their concentration (μg/L) in 6% salted soy whey before and after lactic acid bacterial fermentation.

Compound identification	Identification methods	LRI[a] Ref	LRI[a] Expt	Day 0 Unfermented soy whey	Day 7 *L. plantarum*	Day 7 *L. fermentum*	Day 7 *O. oeni*	Day 7 *T. halophilus*
Acids								
Acetic acid	MS, LRI	1451[b]	1420	61.51 ± 6.00a	69.15 ± 19.27a	251.47 ± 18.31c	44.01 ± 6.84a	188.01 ± 9.54b
Hexanoic acid	MS, LRI	1822[c]	1801	151.74 ± 22.25b	82.16 ± 21.98a	101.66 ± 8.18a	68.17 ± 11.70a	70.22 ± 3.26a
Octanoic acid	MS, LRI	2038[c]	2010	17.95 ± 2.92b	9.01 ± 3.20a	10.38 ± 0.59a	12.79 ± 2.00a	13.44 ± 0.60 ab
Nonanoic acid	MS, LRI	2110[d]	2115	8.96 ± 1.76a	8.80 ± 2.53a	9.63 ± 2.55a	6.44 ± 1.80a	8.09 ± 1.38a
			Subtotal	240.17 ± 26.98	169.12 ± 38.82	373.14 ± 25.60	131.40 ± 17.48	279.76 ± 8.03
Alcohols								
Ethanol	MS			0.00 ± 0.00a	569.74 ± 86.97c	523.79 ± 153.01bc	483.07 ± 124.48bc	329.66 ± 24.88b
1-Pentanol	MS, LRI	1248[e]	1231	26.10 ± 4.62bc	34.11 ± 9.38c	21.84 ± 3.26 ab	22.27 ± 4.56abc	13.73 ± 2.81a
1-Hexanol	MS, LRI	1350[e]	1330	103.97 ± 8.61a	414.96 ± 50.97c	321.39 ± 23.49b	306.78 ± 68.71b	232.88 ± 28.00b
1-Octen-3-ol	MS, LRI	1456[c]	1419	150.36 ± 23.33b	58.57 ± 13.98a	57.67 ± 18.09a	73.82 ± 6.53a	54.35 ± 21.70a
			Subtotal	280.43 ± 31.37	1077.38 ± 119.07	924.70 ± 153.28	885.94 ± 191.85	630.62 ± 64.63
Aldehydes								
Hexanal	MS, LRI	1078[b]	1057	405.94 ± 54.53b	2.57 ± 0.44a	2.17 ± 0.59a	1.43 ± 0.37a	1.31 ± 0.26a
2-Hexenal	MS, LRI	1221[c]	1197	48.04 ± 8.46b	1.46 ± 0.81a	0.48 ± 0.08a	0.95 ± 0.43a	2.81 ± 0.55a
2-Heptenal	MS, LRI	1324[c]	1298	27.10 ± 6.83b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
2-Octenal	MS, LRI	1429[c]	1401	11.37 ± 1.61b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Furfural	MS, LRI	1452[e]	1447	7.41 ± 1.58c	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	4.06 ± 0.81b
Benzaldehyde	MS, LRI	1530[c]	1506	13.59 ± 0.95d	4.80 ± 0.31bc	2.18 ± 1.41a	5.84 ± 1.56c	2.93 ± 0.91 ab
			Subtotal	513.45 ± 67.73	8.46 ± 0.83	4.82 ± 1.55	8.23 ± 1.71	11.11 ± 1.37
Ketones								
β-Damascenone	MS, LRI	1857[f]	1785	24.80 ± 2.64b	25.42 ± 3.18b	24.16 ± 3.04b	22.95 ± 3.80b	11.24 ± 0.66a
			Subtotal	24.80 ± 2.64	25.42 ± 3.18	24.16 ± 3.04	22.95 ± 3.80	11.24 ± 0.66
Volatile phenols								
4-Ethylphenol	MS, LRI	2142[f]	2136	0.00 ± 0.00a	4.90 ± 0.93b	0.00 ± 0.00a	7.34 ± 2.28c	0.00 ± 0.00a
			Subtotal	0.00 ± 0.00	4.90 ± 0.93	0.00 ± 0.00	7.34 ± 2.28	0.00 ± 0.00
			Total	1058.85 ± 105.62	1285.28 ± 140.98	1326.82 ± 164.96	1055.85 ± 203.02	932.73 ± 66.49

a, b, c, d: Statistical analysis using one-way ANOVA at 95% confidence interval (n = 4) and Tukey's post-hoc test. Mean values in the same row with different lower-case letters are significantly different (p < 0.05).

Concentration was determined using butyl butyryl lactate as an internal standard.

[a] LRI: Linear retention index.

[b] Li et al. (2021).

[c] Chua et al. (2018).

[d] Comuzzo et al. (2006).

[e] Ohata et al. (2017).

[f] López et al. (1999).

3-methylbutanoate and ethyl 2-methylbutanoate that have sweet and fruity notes are considered to be important volatile compounds in soy sauce. Esters generation is often related to the metabolism of yeast in soy sauce fermentation. In our previous study, various esters were generated after wine yeast and soy sauce yeasts fermentation (Zhou et al., 2022). These esters include 2-phenethyl acetate, isoamyl acetate and ethyl hexanoate, which are typical aroma compounds in soy sauce (Diez-Simon et al., 2020). Moreover, yeasts metabolize amino acids to generate fusel aldehydes and fusel alcohols such as 2-phenylacetaldehyde and isoamyl alcohol through the Ehrlich pathway, which are confirmed as characteristic volatiles in yeast-fermented salted soy whey (Zhou et al., 2022). Therefore, to develop a soy sauce-like condiment, LAB fermentation alone may not be enough. Yeast fermentation and thermal treatment are essential steps that contribute to the formation of key soy sauce aroma compounds.

3.5. Principal component analysis

To understand the relationship between salt level, LAB species and the formation of volatile compounds, PCA (Fig. 4) was performed based on the calculated concentration (μg/L) of volatile compounds presented in Tables 2 and 3. PC 1 and PC 2 accounted for 47.2% and 21.2% of the variance, respectively. According to the PCA score plot (Fig. 4a), the unfermented salted soy whey samples with a characteristic volatile compound of hexanal, 2-hexenal and 2-heptenal are well separated from LAB-fermented soy whey and positioned in the negative region of PC 1. This result indicates that LAB fermentation greatly changed the volatile profile of salted soy whey. Although *T. halophilus* fermented soy whey with 2% salt is also located at the negative region of PC 1, it is closer to other fermented soy whey samples than to unfermented soy whey samples. The soy whey fermented with *L. fermentum* (at both 2% and 6% NaCl) and *T. halophilus* (at 6% NaCl) were near each other in the same quadrant, suggesting a similar volatile profile shared by these two LAB. This grouping was related to the high concentration of acetic acid (Fig. 4b). On the other hand, *L. plantarum* and *O. oeni* were differentiated from all other samples and located in the first quadrant. It is shown that *L. plantarum* and *O. oeni* fermented soy whey had a characteristic volatile compound of 4-ethylphenol. This agrees with the observed volatile phenol generation discussed above. Salt affected the volatile profile of *T. halophilus* remarkably. At a higher salt content, *T. halophilus* was more like *L. fermentum* while at 2% salt, *T. halophilus* was more similar to unfermented soy whey samples in terms of volatile profiles.
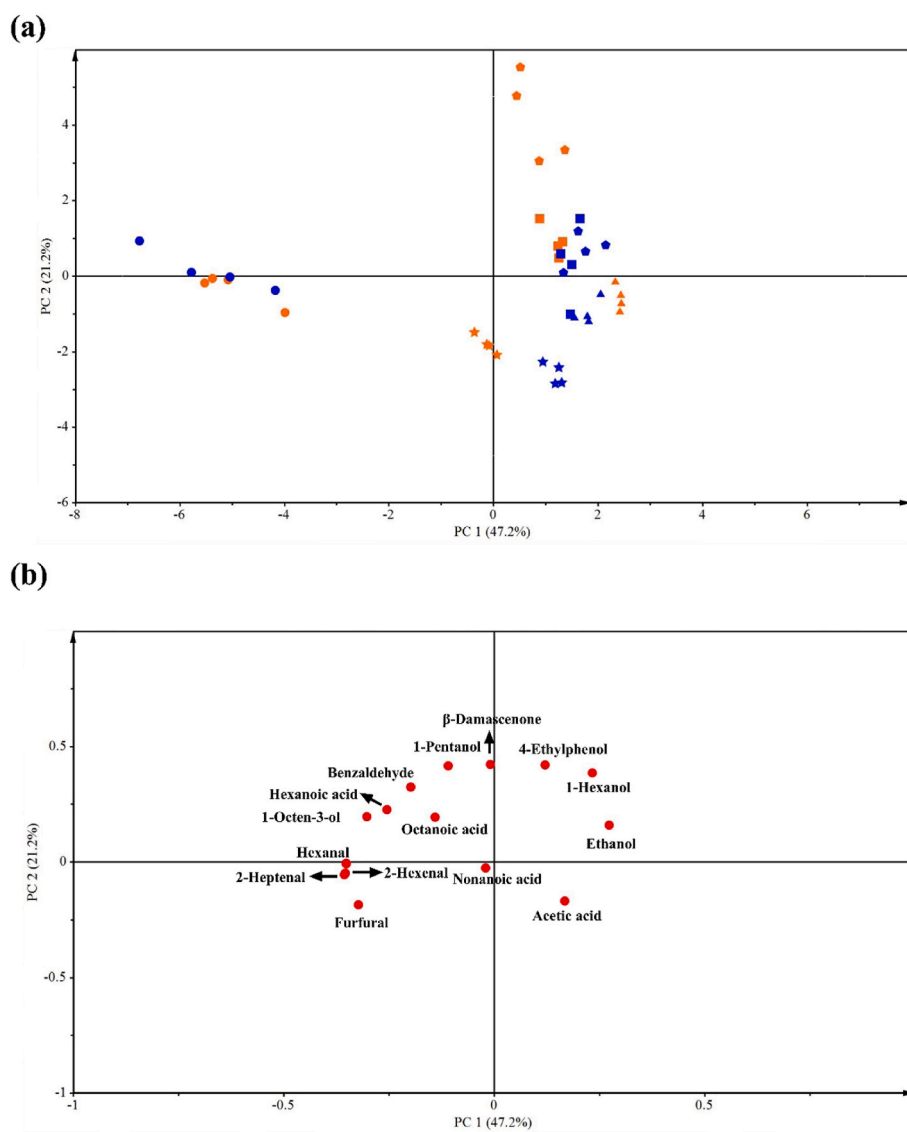

Fig. 4. Principal component analysis of volatiles in unfermented and LAB fermented salted soy whey: (a) score plot showing the distribution of samples; (b) loading plot illustrating the contribution of volatile compounds. (●) unfermented soy whey; (⬟) *L. plantarum*; (▲) *L. fermentum*; (■) *O. oeni*; (★) *T. halophilus*. Symbols in orange represent respective LAB in soy whey with 2% salt while symbols in blue represent that in 6% salt. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Conclusion

Four LAB were used in this study to ferment soy whey at 2 salt levels. Higher salt content exerted an inhibitory effect on the growth, pH reduction, lactic acid generation and amino acids metabolism of *L. plantarum*, *L. fermentum* and *O. oeni*. On the other hand, halophilic *T. halophilus* preferred to grow in soy whey with a higher salt content (6%). Therefore, better cell growth, greater pH reduction, more lactic acid and acetic acid generation and a distinct volatile profile were observed in *T. halophilus* fermented soy whey with 6% NaCl. All four LAB (except for *T. halophilus* at 2% NaCl) utilized fructose at both 2% and 6% salt while *L. plantarum*, *L. fermentum* and *O. oeni* consumed glucose at a lower salt level. In addition, *L. plantarum* and *O. oeni* generated significantly more lactic acid while *L. fermentum* and *T. halophilus* possess greater abilities in producing acetic acid. *L. fermentum* and *T. halophilus* also shared similar volatile profiles with acetic acid as the predominant compounds. LAB fermentation largely diminished the green and beany off-odour by converting aldehydes to alcohols and acids, effectively improving the fermented soy whey aroma compound profile. Therefore, LAB fermentation has the potential to be applied in soy sauce-like condiment development.

CRediT authorship contribution statement

Rebecca Yinglan Zhou: Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft. **Xin Huang:** Data curation, Formal analysis, Visualization, Writing – original draft. **Zhihao Liu:** Data curation, Formal analysis, Visualization, Writing – original draft. **Jian-Yong Chua:** Conceptualization, Methodology, Validation, Visualization, Project administration, Writing – review & editing, Supervision. **Shao-Quan Liu:** Conceptualization, Resources, Project administration, Validation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Bekal, S., Divès, C., Prévost, H., 1998. Citrate lyases of lactic acid bacteria. Lait 78 (1), 3–10.
Chua, J.-Y., Liu, S.-Q., 2019. Soy whey: more than just wastewater from tofu and soy protein isolate industry. Trends Food Sci. Technol. 91, 24–32.
Chua, J.-Y., Lu, Y., Liu, S.-Q., 2017. Biotransformation of soy whey into soy alcoholic beverage by four commercial strains of *Saccharomyces cerevisiae*. Int. J. Food Microbiol. 262, 14–22.
Chua, J.-Y., Lu, Y., Liu, S.-Q., 2018. Evaluation of five commercial non-*Saccharomyces* yeasts in fermentation of soy (tofu) whey into an alcoholic beverage. Food Microbiol. 76, 533–542.
Chua, J.-Y., Tan, S.J., Liu, S.-Q., 2020. Understanding the interaction of isoleucine paired with other amino acids in soy whey alcohol fermentation using *Torulaspora delbrueckii*. Int. J. Food Microbiol. 333, 108802.
Chun, B.H., Han, D.M., Kim, H.M., Park, D., Jeong, D.M., Kang, H.A., Jeon, C.O., 2021. Metabolic features of ganjang (a Korean traditional soy sauce) fermentation revealed by genome-centered metatranscriptomics. mSystems 6 (4) e00441-21.
Chun, B.H., Han, D.M., Kim, K.H., Jeong, S.E., Park, D., Jeon, C.O., 2019. Genomic and metabolic features of *Tetragenococcus halophilus* as revealed by pan-genome and transcriptome analyses. Food Microbiol. 83, 36–47.
Comuzzo, P., Tat, L., Tonizzo, A., Battistutta, F., 2006. Yeast derivatives (extracts and autolysates) in winemaking: release of volatile compounds and effects on wine aroma volatility. Food Chem. 99 (2), 217–230.
De Angelis, M., Gobbetti, M., 2004. Environmental stress responses in *Lactobacillus*: a review. Proteomics 4 (1), 106–122.
Devanthi, P.V.P., Gkatzionis, K., 2019. Soy sauce fermentation: microorganisms, aroma formation, and process modification. Food Res. Int. 120, 364–374.
Devanthi, P.V.P., Linforth, R., Onyeaka, H., Gkatzionis, K., 2018. Effects of co-inoculation and sequential inoculation of *Tetragenococcus halophilus* and *Zygosaccharomyces rouxii* on soy sauce fermentation. Food Chem. 240, 1–8.
Diez-Simon, C., Eichelsheim, C., Mumm, R., Hall, R.D., 2020. Chemical and sensory characteristics of soy sauce: a review. J. Agric. Food Chem. 68 (42), 11612–11630.
Dudley, E.G., Steele, J.L., 2005. Succinate production and citrate catabolism by Cheddar cheese nonstarter lactobacilli. J. Appl. Microbiol. 98 (1), 14–23.
Fei, Y., Liu, L., Liu, D., Chen, L., Tan, B., Fu, L., Li, L., 2017. Investigation on the safety of *Lactobacillus amylolyticus* L6 and its fermentation properties of tofu whey. LWT (Lebensm.-Wiss. & Technol.) 84, 314–322.
Fernández, M., Zúñiga, M., 2006. Amino acid catabolic pathways of lactic acid bacteria. Crit. Rev. Microbiol. 32 (3), 155–183.
Gänzle, M.G., 2015. Lactic metabolism revisited: metabolism of lactic acid bacteria in food fermentations and food spoilage. Curr. Opin. Food Sci. 2, 106–117.
Gao, P., Li, L., Xia, W., Xu, Y., Liu, S., 2020. Valorization of Nile tilapia (*Oreochromis niloticus*) fish head for a novel fish sauce by fermentation with selected lactic acid bacteria. LWT (Lebensm.-Wiss. & Technol.) 129, 109539.
Gürtler, M., Gänzle, M.G., Wolf, G., Hammes, W.P., 1998. Physiological diversity among strains of *Tetragenococcus halophilus*. Syst. Appl. Microbiol. 21 (1), 107–112.
Hahne, H., Mäder, U., Otto, A., Bonn, F., Steil, L., Bremer, E., Hecker, M., Becher, D., 2010. A comprehensive proteomics and transcriptomics analysis of *Bacillus subtilis* salt stress adaptation. J. Bacteriol. 192 (3), 870–882.
He, G., Deng, J., Wu, C., Huang, J., 2017a. A partial proteome reference map of *Tetragenococcus halophilus* and comparative proteomic and physiological analysis under salt stress. RSC Adv. 7 (21), 12753–12763.
He, G., Wu, C., Huang, J., Zhou, R., 2017b. Effect of exogenous proline on metabolic response of *Tetragenococcus halophilus* under salt stress. J. Microbiol. Biotechnol. 27 (9), 1681–1691.
He, G., Wu, C., Huang, J., Zhou, R., 2017c. Metabolic response of *Tetragenococcus halophilus* under salt stress. Biotechnol. Bioproc. Eng. 22 (4), 366–375.
Justé, A., Lievens, B., Frans, I., Marsh, T.L., Klingeberg, M., Michiels, C.W., Willems, K.A., 2008. Genetic and physiological diversity of *Tetragenococcus halophilus* strains isolated from sugar-and salt-rich environments. Microbiology 154 (9), 2600–2610.
Justé, A., Van Trappen, S., Verreth, C., Cleenwerck, I., De Vos, P., Lievens, B., Willems, K. A., 2012. Characterization of *Tetragenococcus* strains from sugar thick juice reveals a novel species, *Tetragenococcus osmophilus* sp. nov., and divides *Tetragenococcus halophilus* into two subspecies, *T. halophilus* subsp. *halophilus* subsp. nov. and *T. halophilus* subsp. *flandriensis* subsp. nov. Int. J. Syst. Evol. Microbiol. 62 (1), 129–137.
Kong, Y., Zhang, L.-L., Zhang, Y.-Y., Sun, B.-G., Sun, Y., Zhao, J., Chen, H.-T., 2018. Evaluation of non-volatile taste components in commercial soy sauces. Int. J. Food Prop. 21 (1), 1854–1866.
Le Marrec, C., Bon, E., Lonvaud-Funel, A., 2007. Tolerance to high osmolality of the lactic acid bacterium *Oenococcus oeni* and identification of potential osmoprotectants. Int. J. Food Microbiol. 115 (3), 335–342.
Li, X., Lee, P.-R., Taniasuri, F., Liu, S.-Q., 2021. Effect of lactic acid bacterial fermentation on amino acids and volatile compounds of pork trimming hydrolysate. Int. J. Food Sci. Technol. 56 (1), 429–440.
Li, X., Liu, S.-Q., 2021. Effect of co-inoculation and sequential inoculation of *Lactobacillus fermentum* and *Pichia kluyveri* on pork hydrolysates fermentation. Food Biosci. 44, 101400.
Liu, B., Li, Y., Cao, Z., Wang, C., 2021. Effect of *Tetragenococcus halophilus*, *Zygosaccharomyces rouxii*, and *Torulopsis versatilis* addition sequence on soy sauce fermentation. Innovat. Food Sci. Emerg. Technol. 69, 102662.
Liu, L., Si, L., Meng, X., Luo, L., 2015. Comparative transcriptomic analysis reveals novel genes and regulatory mechanisms of *Tetragenococcus halophilus* in response to salt stress. J. Ind. Microbiol. Biotechnol. 42 (4), 601–616.
Lonvaud-Funel, A., 1999. Lactic acid bacteria in the quality improvement and depreciation of wine. Lactic Acid Bacteria: Genet. Metabol. Appl. 317–331.
López, R., Ferreira, V., Hernandez, P., Cacho, J.F., 1999. Identification of impact odorants of young red wines made with Merlot, Cabernet Sauvignon and Grenache grape varieties: a comparative study. J. Sci. Food Agric. 79 (11), 1461–1467.
Luh, B.S., 1995. Industrial production of soy sauce. J. Ind. Microbiol. 14 (6), 467–471.
Mbye, M., Baig, M.A., AbuQamar, S.F., El-Tarabily, K.A., Obaid, R.S., Osaili, T.M., Al-Nabulsi, A.A., Turner, M.S., Shah, N.P., Ayyash, M.M., 2020. Updates on understanding of probiotic lactic acid bacteria responses to environmental stresses and highlights on proteomic analyses. Compr. Rev. Food Sci. Food Saf. 19 (3), 1110–1124.
Ohata, M., Zhou, L., Higuchi, K., Nagai, T., Kasamatsu, H., Arihara, K., 2017. Investigation of volatile components and identification of the most potent odour-active component in fermented meat sauce. Flavour Fragrance J. 32 (3), 171–177.
Roling, W., van Verseveld, H.W., 1996. Characterization of *Tetragenococcus halophilus* populations in Indonesian soy mash (kecap) fermentation. Appl. Environ. Microbiol. 62 (4), 1203–1207.
Silva, I., Campos, F.M., Hogg, T., Couto, J.A., 2011. Wine phenolic compounds influence the production of volatile phenols by wine-related lactic acid bacteria. J. Appl. Microbiol. 111 (2), 360–370.
Singracha, P., Niamsiri, N., Visessanguan, W., Lertsiri, S., Assavanig, A., 2017. Application of lactic acid bacteria and yeasts as starter cultures for reduced-salt soy sauce (moromi) fermentation. LWT (Lebensm.-Wiss. & Technol.) 78, 181–188.
Tu, C., Azi, F., Huang, J., Xu, X., Xing, G., Dong, M., 2019. Quality and metagenomic evaluation of a novel functional beverage produced from soy whey using water kefir grains. LWT (Lebensm.-Wiss. & Technol.) 113, 108258.
Udomsil, N., Rodtong, S., Choi, Y.J., Hua, Y., Yongsawatdigul, J., 2011. Use of *Tetragenococcus halophilus* as a starter culture for flavor improvement in fish sauce fermentation. J. Agric. Food Chem. 59 (15), 8401–8408.

Vong, W.C., Liu, S.-Q., 2018. Bioconversion of green volatiles in okara (soybean residue) into esters by coupling enzyme catalysis and yeast (*Lindnera saturnus*) fermentation. Appl. Microbiol. Biotechnol. 102 (23), 10017–10026.

Vrancken, G., Rimaux, T., Wouters, D., Leroy, F., De Vuyst, L., 2009. The arginine deiminase pathway of *Lactobacillus fermentum* IMDO 130101 responds to growth under stress conditions of both temperature and salt. Food Microbiol. 26 (7), 720–727.

Wakinaka, T., Iwata, S., Takeishi, Y., Watanabe, J., Mogi, Y., Tsukioka, Y., Shibata, Y., 2019. Isolation of halophilic lactic acid bacteria possessing aspartate decarboxylase and application to fish sauce fermentation starter. Int. J. Food Microbiol. 292, 137–143.

Wong, K.H., Abdul Aziz, S., Mohamed, S., 2008. Sensory aroma from Maillard reaction of individual and combinations of amino acids with glucose in acidic conditions. Int. J. Food Sci. Technol. 43 (9), 1512–1519.

Xiong, T., Li, J., Liang, F., Wang, Y., Guan, Q., Xie, M., 2016. Effects of salt concentration on Chinese sauerkraut fermentation. LWT–Food Sci. Technol. 69, 169–174.

Xu, D., Wang, W., Wang, P., Zhang, X., Zhang, J., Xu, C., Wang, F., 2021. Soy whey as a promising substrate in the fermentation of soy sauce: a study of microbial community and volatile compounds. Int. J. Food Sci. Technol. 56 (11), 5799–5811.

Yang, X., Hu, W., Jiang, A., Xiu, Z., Ji, Y., Guan, Y., Yang, X., 2019. Effect of salt concentration on quality of Chinese northeast sauerkraut fermented by *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. Food Biosci. 30, 100421.

Zhao, S., Zhang, Q., Hao, G., Liu, X., Zhao, J., Chen, Y., Zhang, H., Chen, W., 2014. The protective role of glycine betaine in *Lactobacillus plantarum* ST-III against salt stress. Food Control 44, 208–213.

Zhou, R.Y., Chua, J.-Y., Liu, S.-Q., 2022. Exploring the feasibility of biotransforming salted soy whey into a soy sauce-like condiment using wine yeast *Torulaspora delbrueckii* and soy sauce yeasts *Zygosaccharomyces rouxii* and *Candida versatilis* as single starter cultures. Food Res. Int. 156, 111350.