https://doi.org/10.17221/246/2021-CJFS

Tetragenococcus halophilus, *Staphylococcus xylosus* and *Staphylococcus saprophyticus* for sardine fermentation

Zhijia Fang[1], Wenjuan Zhu[1], Ying Liu[1], Xinran Wang[1], Yaling Wang[1], Yanyan Wu[2]*

[1]*College of Food Science and Technology, Guangdong Provincial Key Laboratory of Aquatic Product Processing and Safety, Guangdong Provincial Engineering Technology Research Center of Marine Food, Key Laboratory of Advanced Processing of Aquatic Products of Guangdong Higher Education Institution, Guangdong Ocean University, Zhanjiang, China*

[2]*Key Laboratory of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs of the People's Republic of China, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Guangzhou, China*

**Corresponding author: liuying@gdou.edu.cn*

Citation: Fang Z., Zhu W.-J., Liu Y., Wang X.-R., Wang Y., Wu Y. (2023): *Tetragenococcus halophilus*, *Staphylococcus xylosus* and *Staphylococcus saprophyticus* for sardine fermentation. Czech J. Food Sci., 41: 271–278.

Abstract: To improve the quality and enrich the flavour of fermented sardine, *Tetragenococcus halophilus*, *Staphylococcus xylosus*, and *Staphylococcus saprophyticus* were used as a mixed starter for sardine fermentation. And their proportions were optimised using response surface methodology (RSM). The highest sensory score was obtained when the proportions of *T. halophilus*, *S. xylosus*, and *S. saprophyticus* were 1 : 1 : 1. The optimised fermented sardine had the lowest levels of histamine content (0.0190 μg·g^{-1}), total volatile basic nitrogen (TVBN, 208 μg·g^{-1}), and was richer in volatile compounds (308). The results may provide important evidence that *T. halophilus*, *S. xylosus*, and *S. saprophyticus* may be satisfactorily used as a mixed starter to improve the quality and flavour of fermented sardines.

Keywords: fermented products; food fermentative microbiology; mixed starter cultures; sardine products

Sardines are among the most popular edible fish, characterised by their freshness, tenderness, and rapid growth. Long-term preservation deteriorates the quality and flavour of sardine. This dramatically reduces their acceptability and value (Kilinc et al. 2008). Fish fermentation is an important and effective method for fish preservation worldwide; furthermore, the fermentation process could improve the quality and integrity of the fish, resulting in distinctive flavours and extended shelf-life (Gelman et al. 2000). Starter cultures play an important role in fish fermentation (Rhee et al. 2011).

In our previous studies, 158 strains were isolated from fermented Meixiang fish. Three isolates (*Tetragenococcus halophilus*, *Staphylococcus xylosus*, and *Staphylococcus saprophyticus*) showed high salt resistance and antibacterial activity and did not produce histamine (Zhu et al. 2016). As the predominant genera in naturally fermented cassava fish, it was determined that *Staphylococcus* spp. was found to initiate fermentation, and their presence persisted until the end of the fermentation (Anihouvi et al. 2007). As a starter bacterium, *T. halophilus* was also found to increase fish

Supported by China Agriculture Research System (Project No. CARS-47).

https://doi.org/10.17221/246/2021-CJFS

sauce's flavour and reduce the dimethyl disulfide content (Natteewan et al. 2011). *Pediococcus pentosaceus* can also increase the springiness and chewiness sensation to improve the texture of fish (Riebroy et al. 2008). However, no information on applying these three bacterial strains as mixed starter cultures in sardine fermentation is available. Furthermore, their combined effects on fermented sardines' sensory characteristics, texture, and flavour remain unknown. Therefore, this study was conducted to use *T. halophilus*, *S. xylosus*, and *S. saprophyticus* as a mixed starter culture, to use a response surface methodology to optimise their ratio in the starter culture, and to evaluate their effects on the sensory characteristics, texture, and flavour of fermented sardines.

MATERIAL AND METHODS

Material. In April, forty-eight live sardines (*Sardinella aurita*, ~120 g) were purchased from Yanhui Frozen Foodstuffs Co. (Zhanjiang, China). Salt (sea salt, NaCl ≥ 99%, no potassium ferrocyanide) was purchased from Guangdong Province Salt Industry Group Company Limited (Zhongshan, China). Gifu anaerobic medium (GAM medium) was purchased from Nissui Pharmaceutical Co. (Tokyo, Japan).

Starter cultures. *T. halophilus* CAMT 20661 (NCBI accession No. KP_845287), *S. xylosus* CAMT 29661 (NCBI accession No. KP_845286), and *S. saprophyticus* CAMT 29662 (NCBI accession No. KP_845285) were isolated from fermented Meixiang fish and identified with 16 s rDNA sequence in our previous study (Zhu et al. 2016). *T. halophilus, S. xylosus*, and *S. saprophyticus* were grown in GAM medium at 30 °C for 24 h, then harvested, washed, and resuspended with saline water (0.9% NaCl) and adjusted to 10^8 CFU·mL^{-1} (CFU – colony forming unit).

Antagonism test. The metabolic activities of *T. halophilus*, *S. xylosus*, and *S. saprophyticus* and their competitive interactions were analysed using the cross-streak experiments as previously described (Tóth et al. 2013). Bacteria were gradient diluted and streaked onto GAM medium, incubated at 30 °C for 48 h, and then the inhibitory effects of bacteria on the growth of others were recorded.

Fermentation of sardine. Fermentation of sardine was performed as previously described (Achinewhu et al. 2004). Sardines were washed, gutted, gills, and scaled, then soaked in salt at 5 : 1 (fish: salt, *w*/*w*). The samples were inoculated with *T. halophilus*, *S. xylosus*, *S. saprophyticus* and incubated at 20 °C for 48 h (Table 1).

Experimental design for inoculating level of starters in sardine fermentation. To optimise the bacterial ratios in the starter culture for sardine fermentation. The *T. halophilus*, *S. xylosus*, and *S. saprophyticus* (0, 1×10^7, 2×10^7 CFU·g^{-1} fish) were inoculated onto fish for fermentation (Table 1).

Table 1. Groups and starter cultures

Groups	*T. halophilus* (CFU·g^{-1})	*S. xylosus* (CFU·g^{-1})	*S. saprophyticus* (CFU·g^{-1})
Control	–	–	–
1	2×10^7	1×10^7	2×10^7
2	1×10^7	2×10^7	2×10^7
3	–	–	1×10^7
4	1×10^7	–	2×10^7
5	1×10^7	–	–
6	–	1×10^7	2×10^7
7	2×10^7	–	1×10^7
8	2×10^7	1×10^7	–
9	–	1×10^7	–
10	2×10^7	2×10^7	1×10^7
11	1×10^7	2×10^7	–
12	–	2×10^7	1×10^7
13	1×10^7	1×10^7	1×10^7
14	1×10^7	1×10^7	1×10^7
15	1×10^7	1×10^7	1×10^7

CFU – colony forming unit

https://doi.org/10.17221/246/2021-CJFS

Fermented sardine sensory evaluation. Sensory evaluation of fermented fish was conducted by 10 panellists from the Institute of seafood quality centre. A well-trained sensory evaluation team consists of 10 sensory evaluators (5 males and 5 females, ages ranging from 20 to 50 years old) from the College of Food Science and Technology, Guangdong Ocean University (Zhanjiang, China). The sensory panel members were trained for 4 days before the formal sensory evaluation. The sensory panel members were trained for 4 days before the formal sensory evaluation. The samples were cut from the fermented fish and then kept at 20 °C for sensory assessment, lasting 1–2 h. The indexes observed differences in the appearance, odour, flavour, and texture of fermented fish. The sensory items were to describe the intensity of each attribute for fermented fish using an unstructured scale (0–100) as previously described (Shaviklo et al. 2010).

Texture profile analysis. The fermented sardine tissues (length, width, and height, 20 × 20 × 10 mm) were cut, and their texture profile analysis was performed with a rheometer equipped with a 5 mm cylindrical probe (RE 2-33005 S, Yamaden, Japan) as previously described (Wiles et al. 2004). The test speed was 1 mm·s^{-1}, and each analysis was conducted in triplicate assays.

Determination of volatile composition. The volatile compounds of fermented sardines were determined using a Gas Chromatography-Mass Spectrometer (GC-MS, Trace DSQ; Thermo Fisher, USA) as previously described (Hirano et al. 1992). Five g fermented sardine was shredded and pre-incubated at 50 °C for 30 min, then added into 15 mL of Solid Phase Microextraction (SPME) for 30 min. After extraction, the volatile compounds were analysed using GC-MS. The detailed difference in conditions was oven temperature was programmed at an initial 40 °C for 2 min, and then increased to 120 °C at a rate of 6 °C·min^{-1}, increased to 250 °C at a rate of 10 °C·min^{-1}; ion source temperature at 220 °C; data collection over the m/z range of 33–450 amu.

Determination of pH, water activity, salt, histamine, nitrite, and TVBN. The determination of pH, water activity, salt, histamine, nitrite, and total volatile basic nitrogen (TVBN) was performed as the standard procedure. Briefly, 25 g of fermented sardines were cut and homogenised in 225 mL of sterile saline solution (0.9%) for 30 s, and the pH was determined using a pH meter (B-211; HORIBA, Japan). The water activity of fermented sardine was selected with the equilibrium relative humidity (ERH) method. The salt was determined using a salt meter (B-721; HORIBA, Japan). The histamine was determined with a histamine detection kit (60441; Kikkoman, Japan). The nitrite was determined according to AOAC Official Method. The TVBN was determined as previously described (Goulas and Kontominas 2007). All experiments were conducted in triplicate assays.

Statistical analysis. For the generated 15 experiments, response surface methodology (RSM) was used to determine the response data using Design Expert (version 8.0.7, Stat-Ease Inc. Statease Corporation) and F-test for analysis of variance ANOVA.

RESULTS AND DISCUSSION

Antagonism analysis of the bacteria. Antagonism among *T. halophilus*, *S. xylosus*, and *S. saprophyticus* was analysed by streaking the bacteria across each other onto the GAM plate. After incubation at 30 °C for 48 h, the growth of *T. halophilus*, *S. xylosus*, and *S. saprophyticus* was normal, and the species were non-significantly antagonistic to each other (Figure 1). This suggested that these three bacteria could be used as co-fermentative starters.

Experimental results obtained using various bacterial proportions. The various responses of sardine fermentation to mixed starter cultures with different proportions of bacteria resulted in a desirable sensory score. Among the 15 runs, most indices showed

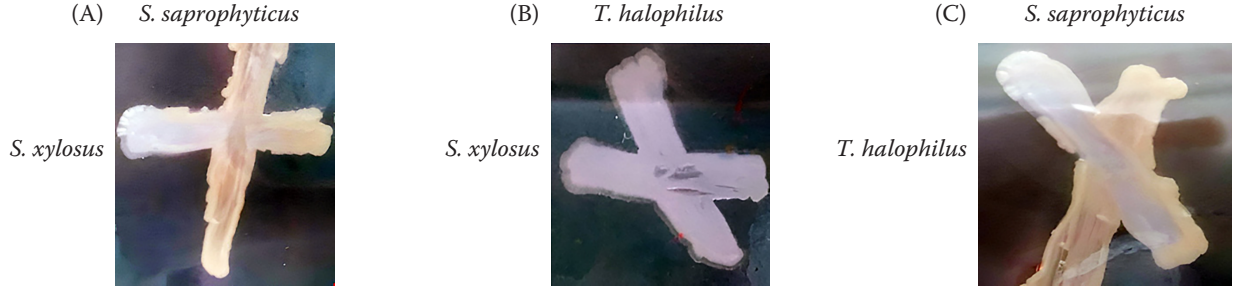

Figure 1. The antagonism between (A) *S. saprophyticus* and *S. xylosus*, (B) *T. halophilus* and *S. xylosus*, (C) *S. saprophyticus* and *T. halophilus*

https://doi.org/10.17221/246/2021-CJFS

a significant improvement. The highest sensory score was obtained from the runs (not including control) in which the proportions of *T. halophilus*, *S. xylosus*, and *S. saprophyticus* were 1 : 1 : 1 (Table 2).

Fitting models. The sensory scores of fermented sardine were fitted to Equation 1 for predicting the quality of sardine using at least square technique (Wanasundara and Shahidi 1996).

$$Y = 2.84 + 3.99X_1 + 2.42X_2 + 3.57X_3 + - 0.48X_1X_2 - 0.25X_1X_3 - 0.27X_2X_3 + - 1.40X_1^2 - 0.66X_2^2 - 1.49X_3^2 \quad (1)$$

The optimum proportions of bacteria in mixed starter cultures were searched for the highest sensory score of fermented sardines. Equation 1 fitted the relationship of coded factors, and variables were obtained using Design Expert. The model, *F*-value for the sensory scores, was 12.91 and R^2 = 0.9587 [see Electronic Supplementary Material (ESM), Table S1]. These results indicate that the model had a high goodness-of-fit and could be employed to predict the optimum proportions of mixed starter cultures for fermented sardines.

3D response surface graphs. The two-factor response surface was employed to optimise the inoculum proportions of starter strains in sardine fermentation, and 3D plots were obtained using the model. Elliptical contours represent the significant interaction between factors, and the centre point represents the optimum values. The effects of the ratios of X_1 (*T. halophilus*) and X_2 (*S. xylosus*) on the sensory score were depicted using 3D response surface graphs (Figure 2). The maximum sensory score was obtained at X_1 = 0.5–1.5 and X_2 = 0.5–2.0; X_1 = 0.5–1.5 and X_3 = 0.5–1.5; X_2 = 0.5–2.0 and X_3 = 0.5–1.5. If the partial derivative of Equation 1 is zero, then the Y is optimised. When X_1 = 1.132, X_2 = 1.208, and X_3 = 0.990, the optimal point Y_{max} = 8.337 was obtained. For simplicity, the optimal proportions of $X_1 : X_2 : X_3$ were set as 1 : 1 : 1 in the next evaluation.

Texture analysis. To explain the ideal sensory properties of fermented sardines with mixed starter culture at optimised ratios, the texture of the 13 groups fermented with starter cultures at different ratios (listed in Table 3 except redundancy runs 14 and 15) and the controls were analysed (Table 4). The springiness values of all 13 groups were higher than those of the controls (620.23 g), and Group 13 had the highest springiness value (1 998.48 g). The breaking force and distance of Group 13 were also greater than those of the groups fermented with a single bacterium. These include Groups 3, 5, and 9 and the controls (2.50 N and 2.95 mm). The results indicated that the mixed starter culture increased the springiness, breaking force, and distance to improve the sensory texture of fermented sardines. Transglutaminase can significantly increase the tensile strength of porcine meat, and it was also produced by *S. xylosus* (Wang et al. 2016).

Table 2. The response surface central composite design results

Run	X1	X2	X3	Sensory score
Control	0	0	0	4.614
1	2	1	2	5.744
2	1	2	2	6.256
3	0	0	1	5.042
4	1	0	2	5.744
5	1	0	0	5.400
6	0	1	2	5.428
7	2	0	1	7.060
8	2	1	0	5.804
9	0	1	0	4.488
10	2	2	1	6.385
11	1	2	0	7.000
12	0	2	1	6.298
13	1	1	1	8.072
14	1	1	1	8.000
15	1	1	1	8.060

https://doi.org/10.17221/246/2021-CJFS

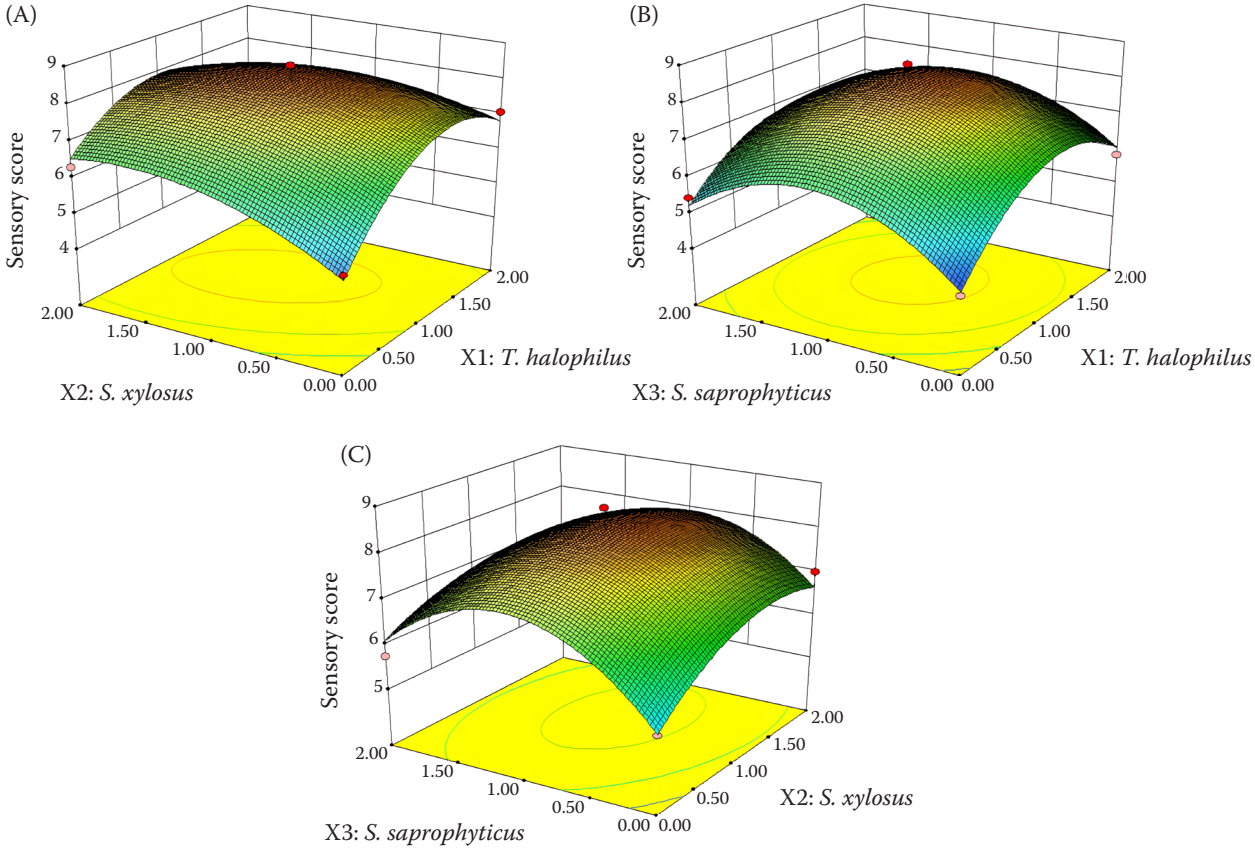

Figure 2. The effect of different inoculation proportions between (A) *T. halophilus* and *S. xylosus*, (B) *T. halophilus* and *S. saprophyticus*, (C) *S. xylosus* and *S. saprophyticus* on sensory score

pH value, water activity, salt, histamine, nitrite, and TVBN contents of fermented sardines. To explain the differences in sensory and texture among all the groups, the pH value, water activity, salt, histamine, nitrite, and TVBN contents of the fermented sardines were tested (Table 4). The pH of sardines from all groups was 6.0–6.2. This was lower than the pH value of 7.1 of the control group, indicating that these mixed starter cultures may produce acid. *T. halophilus* was reported to produce lactic acid and reduce pH in high-saline conditions (Kobayashi et al. 2004). The water activity of sardines in all groups, except Groups 1 and 10, was higher than that of the controls. This indicated that starter cultures maintained water activity during sardine fermentation. The effects of starter cultures on salt and nitrite contents are not irregular or significant, and the nitrate reductase of bacteria contributes to a reduction of nitrite.

Table 3. The results of texture

Groups	Breaking force (N)	Breaking distance (mm)
Control	2.50	2.95
1	4.67	5.10
2	4.73	5.45
3	3.11	4.45
4	4.38	5.15
5	3.50	4.80
6	5.12	5.95
7	4.70	5.05
8	4.78	5.10
9	3.13	4.60
10	4.82	5.55
11	4.65	5.20
12	4.73	5.35
13	5.85	7.15

The mixed starter cultures significantly reduced histamine and TVBN contents. Group 13 had the lowest levels of histamine and TVBN (0.190 μg·g^{-1} and 208 μg·g^{-1}, respectively), which were lower than the 7.480 μg·g^{-1} and 468 μg·g^{-1}, respectively, of the control group. The three bacteria inhibited the production of histamine and TVBN during sardine fermentation. *S. xylosus* was reported to reduce the TVBN and histamine levels in fermented sausages (Wang et al. 2016). The high sensory score may be ascribed to the mixed starter cultures and

https://doi.org/10.17221/246/2021-CJFS

Table 4. The main ingredients of fermented fish products

Groups	pH	Water activity	Salt (%)	Histamine (μg·g^{-1})	Nitrite (g)	TVBN (μg·g^{-1})
Control	7.1	0.815	20	7.480	ND	468
1	6.1	0.810	18	2.380	ND	308
2	6.0	0.897	20	0.760	ND	292
3	6.1	0.914	20	4.440	ND	352
4	6.0	0.935	19	4.280	ND	336
5	6.0	0.876	18	1.210	ND	308
6	6.0	0.857	18	1.010	ND	320
7	6.1	0.857	18	2.491	ND	302
8	6.2	0.850	19	2.387	ND	308
9	6.0	0.845	19	3.678	ND	406
10	6.1	0.790	20	1.425	ND	280
11	6.1	0.878	19	1.080	ND	264
12	6.1	0.873	18	3.520	ND	252
13	6.2	0.903	18	0.190	ND	208

ND – not detected; TVBN – total volatile basic nitrogen

their inhibitory activity on histamine and TVBN production, the primary odorant substances.

Volatile compound composition analysis. To further explain the good odour and flavour of sardines after fermentation with the mixed starter cultures of *T. halophilus*, *S. xylosus*, and *S. saprophyticus*, the presence of volatile components in fermented sardines was determined using GC-MS, and the volatile compounds were identified using the NBS/WILEY reference database. Alcohols, aldehydes, ketones, esters, hydrocarbons, acids, ethers, amines, and phenols were the primary compounds found in fermented sardines (Figure 3). Among these groups, Group 13 produced the highest variety of volatiles (308 kinds), which was higher than that produced by the controls (53 types) (Figure 3B). Among these volatile compounds, alcohols, aldehydes, ethers, and hydrocarbons constituted a relatively moderate proportion in Group 13.

Among the alcohol compounds, 2-ethyl cyclobutanol, 1-pentene-3-ol, 2-methylcyclopentanol, and 1-ethyl cyclopropanol are major unsaturated alcohols, responsible for some unique flavours (Silva et al. 1996). A high proportion of aldehydes may also be attributed to *T. halophilus*, which was found to produce 2-methylpropanal, 2-methylbutanal, 3-methylbutanal, and benzaldehyde in fish sauce fermentation (Natteewan et al. 2011). Moreover, *S. xylosus* may contribute less to the production of ketones, esters, and hydrocarbons. *S. xylosus* significantly affects the volatile compounds of Fermented Sausage Sucuk (Kaban and Kaya 2009), which may also explain the lowest sensory score in Group 9, which is only fermented by *S. xylosus*. The compound 1-pentene-3-ol smells like smoked fatty fish and contributes to off-flavour (Iglesias and Medina 2008). The content of 1-pentene-3-ol in Group 5, only fermented by *T. halophilus,* was higher than other unsaturated alcohols. This may contribute to the low odour score. Group 3, which was only fermented by *S. saprophyticus*, also had a low sensory score. This may be due to lipid oxidation induction and aldehydes' production. *S. saprophyticus* has high esterase activity (Gürtler et al. 1998). Among these aldehyde, hexaldehyde and 2-methyl butyraldehyde was detected in Group 13 (accounting for 2.17% and 0.4%, respectively), contributing to the unique flavours of fruity and coffee. Ketones are usually produced from the decomposition of unsaturated lipids, and the production of Ketones depends on lipoxidase activity (Kaban and Kaya 2009). The 2-nonyl ketone and 2-decyl ketone detected in Group 13 are two attractive fragrance components of the Ruta (Dethier 1941). Hydrocarbons have a light, pleasant odour (Bruce and Schmidt 1994) and were the major volatile components of Group 13. Group 13 produced more esters, which usually contribute to the characteristic smell of fruits. *T. halophilus* produces benzaldehyde, reducing dimethyl disulfide in fermented fish sauce(Natteewan et al. 2011). Trimethylamine is responsible for the fishy smell during fish decay (Gallart-Jornet et al. 2007). Trimethylamine was not detected in Group 13. These data indicated that mixed starter cultures of *T. halophilus*, *S. xylosus*, and *S. saprophyticus* increased the flavour varieties and improved the flavour of fermented sardines.

https://doi.org/10.17221/246/2021-CJFS

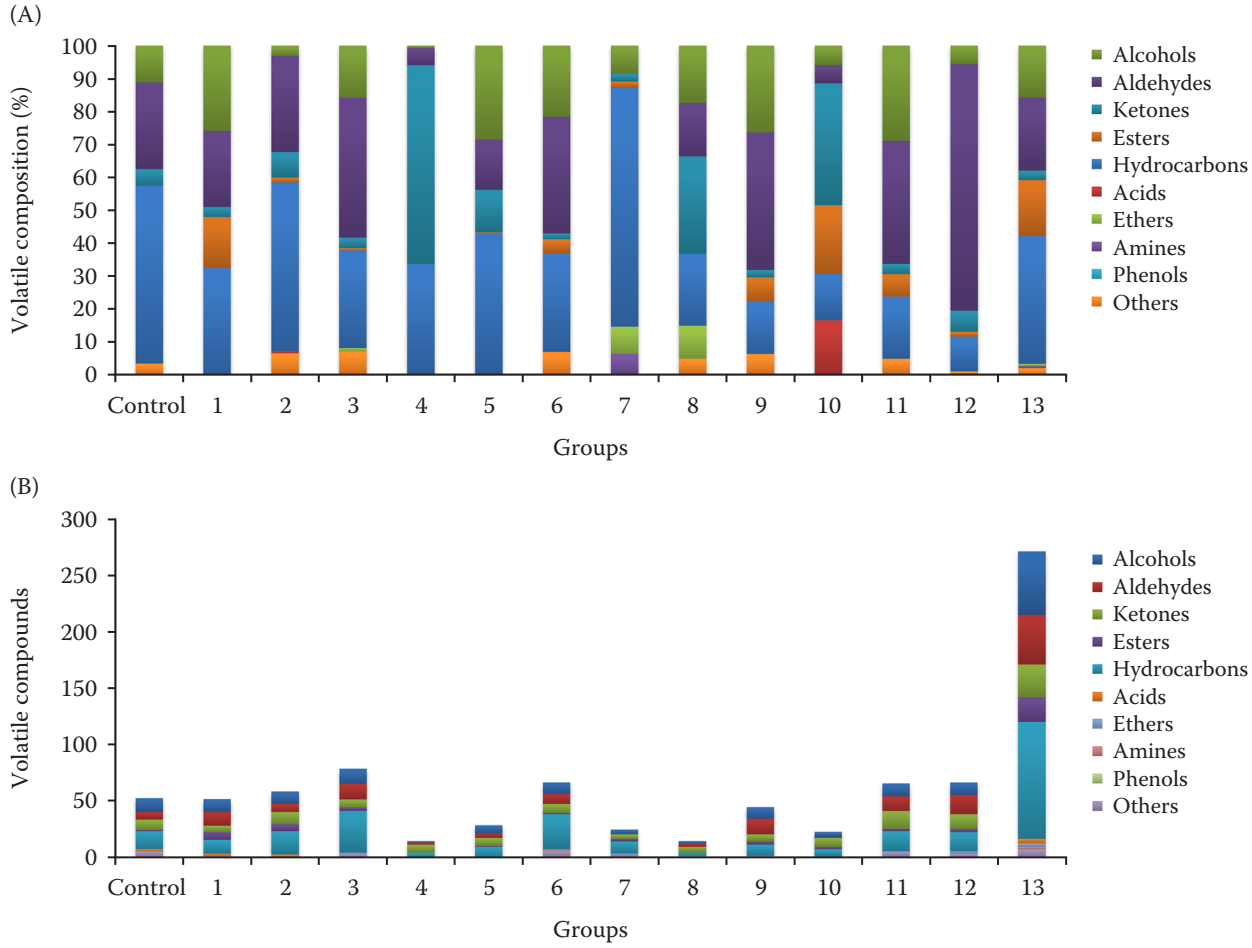

Figure 3. The volatile compounds analysis. Comparison of (A) volatile composition and (B) compounds of sardine fermented with different mixed starter cultures of *T. halophilus*, *S. xylosus*, and *S. saprophyticus*

CONCLUSION

The mixed starter cultures of *T. halophilus*, *S. xylosus*, and *S. saprophyticus* could increase the sensory properties of fermented sardines by improving their texture profiles, reducing histamine and TVBN content, and enriching flavours by producing more volatile compounds, which include alcohols, aldehydes, hydrocarbons, and esters. This work provided a novel mixed starter culture for improving the quality and flavour of fermented sardines.

Acknowledgement. We are grateful to Vikas Narang for his kind assistance with language proofing.

REFERENCES

Achinewhu S.C., Amadi E.N., Barimalaa I.S., Eke J. (2004): Microbiology of naturally fermented fish (*Sardinella* sp.). Journal of Aquatic Food Product Technology, 13: 47–53.

Anihouvi V.B., Sakyi-Dawson E., Ayernor G.S., Hounhouigan J.D. (2007): Microbiological changes in naturally fermented cassava fish (*Pseudotolithus* sp.) for lanhouin production. International Journal of Food Microbiology, 116: 287–291.

Bruce L.G., Schmidt G.W. (1994): Hydrocarbon fingerprinting for application in forensic geology: Review with case studies. AAPG bulletin, 78: 1692–1710.

Dethier V.G. (1941): Chemical factors determining the choice of food plants by Papilio larvae. The American Naturalist, 75: 61–73.

Gallart-Jornet L., Barat J.M., Rustad T., Erikson U., Escriche I., Fito P. (2007): Influence of brine concentration on Atlantic salmon fillet salting. Journal of Food Engineering, 80: 267–275.

Gelman A., Drabkin V., Glatman L. (2000): Evaluation of lactic acid bacteria, isolated from lightly preserved fish products, as starter cultures for new fish-based food products. Innovative Food Science & Emerging Technologies, 1: 219–226.

Goulas A.E., Kontominas M.G. (2007): Combined effect of light salting, modified atmosphere packaging and

https://doi.org/10.17221/246/2021-CJFS

oregano essential oil on the shelf-life of sea bream (*Sparus aurata*): Biochemical and sensory attributes. Food Chemistry, 100: 287–296.

Gürtler M., Gänzle M.G., Wolf G., Hammes W.P. (1998): Physiological diversity among strains of *Tetragenococcus halophilus*. Systematic and Applied Microbiology, 21: 107–112.

Hirano T., Zhang C.-H., Morishita A., Suzuki T., Shirai T. (1992): Identification of volatile compounds in ayu fish and its feeds. Journal of the Japanese Society of Fisheries Science, 58: 547–557.

Iglesias J., Medina I. (2008): Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. Journal of Chromatography A, 1192: 9–16.

Kaban G., Kaya M. (2009): Effects of *Lactobacillus plantarum* and *Staphylococcus xylosus* on the quality characteristics of dry fermented sausage 'Sucuk'. Journal of Food Science, 74: S58–S63.

Kilinc B., Cakli S., Tolasa S. (2008): Quality changes of sardine (*Sardina pilchardus*) patties during refrigerated storage. Journal of Food Quality, 31: 366–381.

Kobayashi T., Kajiwara M., Wahyuni M., Hamada-Sato N., Imada C., Watanabe E. (2004): Effect of culture conditions on lactic acid production of *Tetragenococcus* species. Journal of Applied Microbiology, 96: 1215–1221.

Natteewan U., Sureelak R., Yeung Joon C., Yanglin H., Jirawat Y. (2011): Use of *Tetragenococcus halophilus* as a starter culture for flavor improvement in fish sauce fermentation. Journal of Agricultural & Food Chemistry, 59: 8401–8408.

Rhee S.J., Lee J.-E., Lee C.-H. (2011): Importance of lactic acid bacteria in Asian fermented foods. Microbial Cell Factories, 10: S5.

Riebroy S., Benjakul S., Visessanguan W. (2008): Properties and acceptability of Som-fug, a Thai fermented fish mince, inoculated with lactic acid bacteria starters. LWT – Food Science and Technology, 41: 569–580.

Shaviklo G.R., Arason S., Thorkelsson G., Sveinsdottir K., Martinsdottir E. (2010): Sensory attributes of haddock balls affected by added fish protein isolate and frozen storage. Journal of Sensory Studies, 25: 316–331.

Silva M.L., Malcata F.X., De Revel G. (1996): Volatile contents of grape marcs in Portugal. Journal of Food Composition and Analysis, 9: 72–80.

Tóth E.M., Kériné Borsodi A., Felföldi T., Vajna B., Sipos R., Márialigeti K. (2013): Exercise 69: Detection of antagonism between microbes using cross-streak experiments. In: Tóth E.M., Márialigeti K. (eds): Practical Microbiology: Based on the Hungarian Practical Notes Entitled Mikrobiológiai Laboratóriumi Gyakorlatok. Budapest, Eötvös Loránd Tudományegyetem: 88–90.

Wanasundara P., Shahidi F. (1996): Optimization of hexametaphosphate-assisted extraction of flaxseed proteins using response surface methodology. Journal of Food Science, 61: 604–607.

Wang X., Ren H., Wang W., Xie Z.J. (2016): Effects of a starter culture on histamine reduction, nitrite depletion and oxidative stability of fermented sausages. Journal of Food Safety, 36: 195–202.

Wiles J.L., Green B.W., Bryant R. (2004): Texture profile analysis and composition of a minced catfish product. Journal of Texture Studies, 35: 325–337.

Zhu W., Zhang X., Ren W., Sun L., Wang Y., Liu Y. (2016): Isolation, identification and application of lactic acid bacteria from Chinese traditional fermented sea-fish. Medicine and Biopharmaceutical: 1506–1514.

Received: November 19, 2021
Accepted: July 27, 2023
Published online: August 14, 2023