Dynamics of spoilage bacterial communities in fish cake evaluated by culture-based and culture-independent methods

YING WEI – LI ZHAO – CHUNQING BAI – YONG JIANG – MEILAN YUAN – LILI CHEN

Summary
In order to study the spoilage-related microbiota of fried and boiled fish cakes, a combination of traditional plating, culture-independent and culture-based methods were used to analyse the fish cake stored at 4 °C. The population diversity was studied by selective agar cultivation, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) of DNA directly extracted from fish cake and from bulk cells from plate counting media. No significant difference in spoilage bacteria between fried and boiled fish cakes could be found during storage period. Plate counts on selective agar showed that the major bacterial group was lactic acid bacteria. Flavobacteriaceae, *Psychrobacter* sp. and *Pseudomonas poae* were detected during the entire storage period in DGGE profile. The use of culture-independent method highlighted the species that were not detected by plating. In contrast, culture-based analysis detected bacterial species that were not found in DNA extracted directly from sample. This study confirmed that a combination of traditional plating, culture-independent and culture-based approaches could enhance understanding of the populations of spoilage bacteria.

Keywords
denaturing gradient gel electrophoresis; fish cake; diversity; storage

Fish cake as a highly nutritious fresh water surimi product is widely consumed in China now. The main ingredient for producing fish cakes is fresh water surimi, which is a concentrated myofibrillar protein obtained from mechanically deboned fish flesh. Fish cakes are produced in the following process: starch, egg white and spices are added into surimi, stirring well, and then shaped with mould, followed by cooking (frying or boiling) and then packaged [1, 2]. No disinfection treatment of the raw meat and poor hygiene conditions may cause quality problems. Quality control and potential shelf life of fish cakes are currently still based on assessing total viable counts (TVC) based on Chinese standard GB10132-2005 [3]. However, many studies revealed that not the total numbers of microbiota on seafood are responsible for its spoilage, but only a small fraction of microorganisms, in particular specific spoilage organisms [4, 5]. Thus it is essential to study the spoilage bacteria in fish cake. Bacterial communities of fresh water surimi-based fish cakes have not been studied intensively and the influences of two different heat treatments on the composition of the bacterial flora and shelf life of fresh water surimi-based products have also not been studied intensively before.

Rapid and precise methods for detecting the microorganisms are very important. Conventional planting method is useful for characterization of bacteria, but many kinds of bacteria are unable to grow on the media [4]. As a culture-independent method, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) can be used to analyse the bacterial DNA extracted directly from food samples, which can reveal the presence of non-cultivable bacteria [6, 7]. However, the detection limit of DGGE method varies between 10^3 CFU·g^{-1} and 10^8 CFU·g^{-1} [8]. Thus, microorganisms present at low densities in samples cannot be revealed by the culture-independent method. Alternatively, culture-based

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DGGE analysis can enrich cultivable microorganisms and make them detectable in the DGGE profile. Not every band in the DGGE profile can be sequenced and analysed, which may cause that some important bacteria might remain undetected [9]. Thus the traditional culture-based method can help to reveal some microorganisms that were not found in the DGGE profile. In this sense, a combination using traditional cultivation method, culture-independent and culture-based methods may enhance full characterization of communities of spoilage bacteria.

In the present study, our aim was to characterize microbial populations on fried and boiled fish cakes, using conventional cultivation, culture-based and culture-independent methods. This research will facilitate the development of novel detection methods and improve preservation techniques that target specific spoilage organisms.

MATERIALS AND METHODS

Sample preparation

The fried and boiled fish cakes were prepared in a local meat factory according to the conventional technique without the addition of any preservatives. The fish cake was made with surimi (mechanically deboned silver carp mince), sodium chloride, starch, egg, water and flavour additives. The raw materials were mixed and shaped with moulds and then cooked (fried or boiled). After cooling, the samples were sealed in packages under sterile conditions and stored in a refrigerator at 4 °C ± 1 °C until sampling. The traditional microbiological analyses were performed every day until the end of shelf life. On days 0, 1, 2, 3 of storage for boiled fish cakes, and on days 0, 3, 6, 8 of storage for fried fish cakes, bacterial DNA was extracted from plate count agar plates (bulk cell DNA samples) and directly from the cake samples (direct DNA extraction) to characterize the microbial population.

Microbiological analyses

Samples of 25 g were taken under sterile conditions, mixed with 225 ml of peptone saline and homogenized in a stomacher (Seward, Worthing, United Kingdom) for 3 min. Serial decimal dilutions were prepared in which 1 ml of a suitable dilution was taken and spread in triplicate on following agars:

- plate count agar (PCA) incubated at 30 °C for 72 h for enumeration of total viable counts (TVC),
- de Man, Rogosa and Sharpe (MRS) agar incubated under anaerobic conditions at 30 °C for 48 h for enumeration of lactic acid bacteria (LAB),
- violet red bile agar (VRBA) incubated at 37 °C for 48 h for enumeration of Enterobacteriaceae,
- mannitol salt agar (MSA) incubated at 37 °C for 48 h for enumeration of staphylococci,
- iron agar (IA) incubated at 37 °C for 48 h for enumeration of H_2S-producing bacteria.

All the media were purchased from Hopebio (Qingdao, China).

Extraction of bacterial DNA directly from the samples (direct DNA)

On days 0, 1, 2, 3 for boiled fish cakes, and on days 0, 3, 6, 8 for fried fish cakes, DNA extraction directly from the fish cakes was performed as described by Rudi et al. [10]. DNA extraction was performed by a combination of different centrifugation steps (first centrifugation at low speed to get rid of the solid matter of the samples and then centrifugation at a high speed to sediment the bacteria). An amount of 25 g of the sample was diluted with 80 ml sterilized physiological saline and treated with a stomacher for 2 min. The sample suspension was centrifuged at 300 ×*g* for 5 min at 4 °C. The supernatant was removed and centrifuged at 25 000 ×*g* for 20 min at 4 °C. Then, the pellet was collected and used for DNA extraction with a QIAamp kit (Qiagen, Hilden, Germany).

Collection of bacteria from plate count agar plates (bulk cell DNA)

On days 0, 1, 2, 3 for boiled fish cakes, and on days 0, 3, 6, 8 for fried fish cakes, a complete plate swab, using an inoculation loop, was taken from plate count agar (PCA) plates after counting, placed in a microtube and suspended in 100 μl of TE buffer (Tris-HCl buffer, pH 8.0, containing 1.0 mmol·l^{-1} ethylenediaminetetraacetic acid) for DNA extraction with a QIAamp kit.

PCR-DGGE analysis

Nested polymerase chain reaction (PCR) was used for a more efficient amplification of the V3 region of the 16S rRNA gene extracted from the samples and PCA plates. In the first round of PCR, the universal primers 8f (5'-GGA GAG TTT GAT CAT GGC T-3') and 798r (5'-CCA GGG TAT CTA ATC CTG TT-3') were used to amplify the 16S rRNA gene fragments [11]. The TaKaRa Taq kit (Takara Bio, Kusatsu, Japan) was used to perform PCR. The PCR mix (50 μl per reaction) was composed of 1 × PCR buffer, 200 nmol·l^{-1} each primer, 200 μmol·l^{-1} dNTPs, 1.5 U Taq polymerase,

2 μl of template DNA solution, 1.5 mmol·l^{-1} $MgCl_2$ and double distilled water (ddH_2O). The temperature programme consisted of initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The amplification products obtained from the first round of PCR were then used as templates for the second round of PCR with bacterial DGGE primers GC338f (5'-GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG-3') and 518r (5'-ATT ACC GCG GCT GCT GG-3'). PrimeSTAR Max Premix (Takara Bio) was used in the second PCR reaction. The 25 μl PCR reaction mixture consisted of 1×PrimeSTAR Max Premix buffer, 400 nmol·l^{-1} each primer, 200 μmol·l^{-1} dNTPs, 1 μl of template DNA and ddH_2O. The initial denaturation was performed at 94 °C for 3 min and a "touchdown" PCR was carried out according to the methods of Jiang et al. [12]. All of the PCR products were checked on a 1.2% agarose gel. The PCR Clean-Up System (Tiangen, Beijing, China) was used to purify the PCR products.

The DCode system for DGGE (Bio-Rad, Hercules, California, USA) was used for DGGE analysis of PCR amplicons. The DGGE gel was prepared using 8% polyacrylamide gel, and a denaturing gradient of 35–55% (100% of the denaturing agent was defined as 7 mol·l^{-1} urea and 40% formamide) and a 0.5× TAE buffer (20 mmol·l^{-1} Tris, 10 mmol·l^{-1} acetic acid, 0.5 mmol·l^{-1} EDTA, pH 8.0). Electrophoresis was performed for 16 h at 85 V·cm^{-1} and 60 °C. The gel was then stained with $AgNO_3$ [13] and photographed.

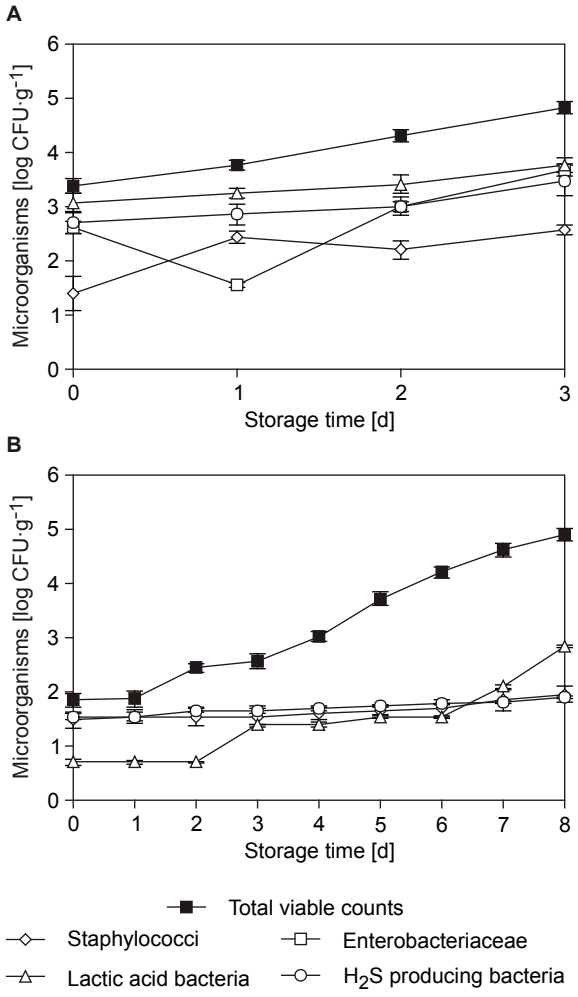

Fig. 1. Microbial growth on fish cakes during storage.

A – Boiled fish cake, B – Fried fish cake.

Sequence of the DGGE fragments and band identification

To determine the microbial populations, the selected DGGE bands were punched from the gel immediately after staining. The bands were transferred into a 30 μl TE buffer (pH 8.0) and stored overnight at 4 °C. A volume of 5 μl of eluate was used as a template and re-amplified with 338f primer (5'-ACT CCT ACG GGA GGC AGC AG-3') and 518f primer. The PCR protocol described above was used. DNA was sequenced by Invitrogen (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and searched in the gene sequence database GenBank (National Center for Biotechnology Information, Bethesda, Maryland, USA) to identify the bacterial species.

RESULTS AND DISCUSSIONS

Microbiological analysis

The end of shelf life was determined at TVC surpassing 50000 CFU·g^{-1} on PCA plates [14]. Thus, the shelf-life of boiled fish cakes and fried fish cakes was eight and three days, respectively. Fig. 1A shows that lactic acid bacteria were dominant in boiled fish cakes, followed by H_2S-producing bacteria, Enterobacteriaceae and staphylococci. With the extension of storage time of the boiled fish cake, numbers of staphylococci increased initially and then decreased slightly, whereas Enterobacteriaceae decreased initially and then increased slightly. This might be attributed to the different effect of symbiosis or antagonism of these microorganisms. Fig. 1B shows that lactic acid bacteria gradually increased and became dominant in the in the final period (7–8 days) of storage of fried fish cakes. The numbers of staphylococci and H_2S-producing bacteria gradually increased with the

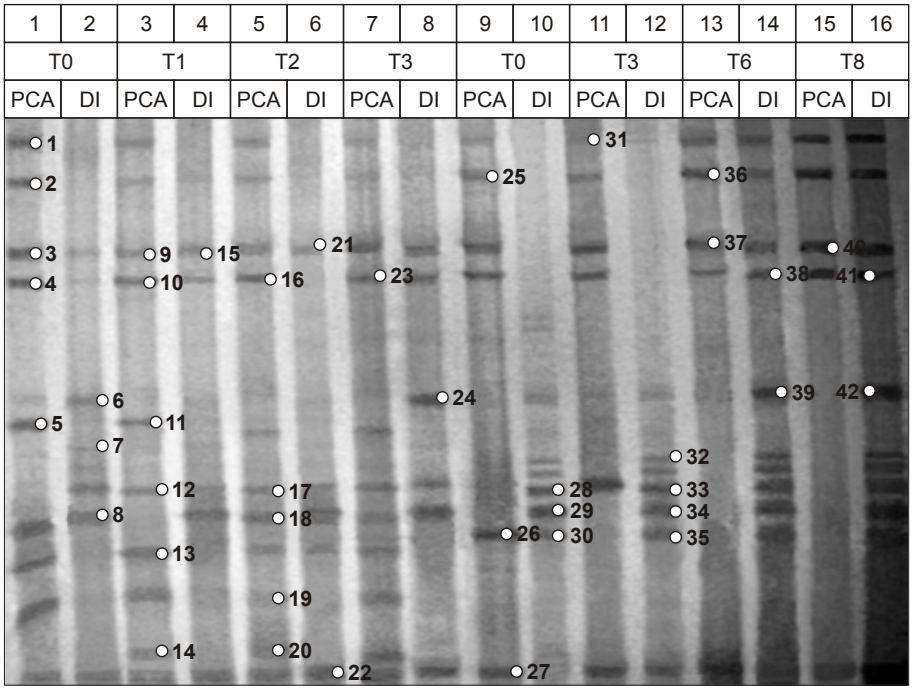

Fig. 2. DGGE bacterial profiles of fried and boiled fish cakes during the storage.

Lanes 1–8 represent boiled fish cakes, lanes 9–16 represent fried fish cakes. Storage time: T0 – 0 days, T1 – 1 day, T2 – 2 days, T3 – 3 days, T6 – 6 days, T8 – 8 days. DI – direct DNA extraction, PCA – bulk cells from PCA plates.

extension of storage time.

Initially (at zero time), TVC of the fried and boiled fish cakes were 1.84 log CFU·g^{-1} and 3.39 log CFU·g^{-1}, respectively (Fig. 1). The initially low microbial levels in fried fish cake might be the reason that the fried fish cakes had longer shelf life than the boiled fish cakes, which suggested that the frying process provided more thorough sterilization of the fish cakes [15]. Applying disinfection treatment (like irradiation or ozone treatment) to raw meat and improving the hygiene conditions might help to reduce the bacterial contamination of the fish cake.

The results in Fig. 1 show that the dominant bacteria causing spoilage of fried and boiled fish cakes were lactic acid bacteria, which is consistent with other studies [16, 17]. The metabolic activity of lactic acid bacteria resulted in spoilage appearing as "sour" off-favours and off-odours, milky exudates and frequently, slimy appearance, swelling of the pack and/or greening [18].

Identification of bacterial community through PCR-DGGE

The bacterial profile of the stored samples changed during the storage period (Fig. 2). The brightness of bands in DGGE profiles represented the numbers of microorganisms, with brighter stripes indicating higher microorganism content. At the beginning of the shelf life, there were fewer bands from the direct DNA method (T0 in Fig. 2 corresponds to the beginning of shelf-life). In the lanes of boiled fish cakes, the bands from the bulk cell method were more complex than the bands from direct DNA method (Fig. 2).

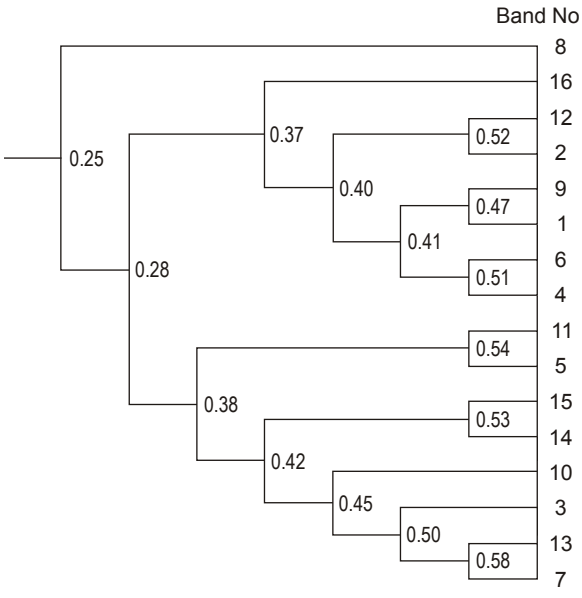

Fig. 3. Cluster analysis of molecular banding patterns generated by DGGE.

Tab. 1. 16S rDNA sequence similarities to closest relatives of DNA recovered from the respective bands in DGGE gel.

Band No	Closest relative in BLAST	Closest phylogenetic relative	Similarity [%]
1	*Pseudomonas synxantha*	NZ_CM001514.1	100
2	*Pseudomonas synxantha*	NZ_CM001514.1	100
3	*Pseudomonas poae*	NC_020209.1	99
4	*Flavobacteriaceae bacterium*	NC_013062.1	94
5	*Flavobacteriaceae bacterium*	NC_013062.1	94
6	*Psychrobacter* sp.	NC_009524.1	100
7	*Psychrobacter* sp.	NC_009524.1	100
8	*Streptococcus lutetiensis*	NC_021900.1	95
9	*Pseudomonas poae*	NC_020209.1	99
10	*Flavobacteriaceae bacterium*	NC_013062.1	97
11	*Flavobacteriaceae bacterium*	NC_013062.1	94
12	*Acinetobacter oleivorans*	NC_014259.1	97
13	*Riemerella anatipestifer*	NC_014738.1	97
14	*Acinetobacter oleivorans*	NC_014259.1	97
15	*Pseudomonas poae*	NC_020209.1	99
16	*Flavobacteriaceae bacterium*	NC_013062.1	97
17	*Staphylococcus aureus*	NC_007795.1	97
18	*Streptococcus aureus*	NC_007795.1	98
19	*Pseudomonas* sp.	NC_019670.1	99
20	*Acinetobacter oleivorans*	NC_014259.1	97
21	*Pseudomonas poae*	NC_020209.1	99
22	*Pseudomonas poae*	NC_020209.1	99
23	*Flavobacteriaceae bacterium*	NC_013062.1	97
24	*Psychrobacter* sp.	NC_009524.1	100
25	*Pseudomonas poae*	NC_020209.1	98
26	*Pseudomonas synxantha*	NZ_CM001514.1	99
27	*Pseudomonas moraviensis*	NZ_CM002330.1	100
28	*Weissella koreensis*	NC_015759.1	96
29	*Weissella koreensis*	NC_015759.1	96
30	*Pseudomonas moraviensis*	NZ_CM002330.1	100
31	*Pseudomonas synxantha*	NZ_CM001514.1	100
32	*Bacillus pumilus*	NC_009848.1	100
33	*Weissella koreensis*	NC_015759.1	99
34	*Weissella koreensis*	NC_015759.1	96
35	*Pseudomonas moraviensis*	NZ_CM002330.1	100
36	*Pseudomonas synxantha*	NZ_CM001514.1	99
37	*Pseudomonas poae*	NC_020209.1	99
38	*Flavobacteriaceae bacterium*	NC_013062.1	94
39	*Psychrobacter* sp.	NC_009524.1	98
40	*Pseudomonas poae*	NC_020209.1	98
41	*Flavobacteriaceae bacterium*	NC_013062.1	97
42	*Psychrobacter* sp.	NC_009524.1	98

BLAST – Basic Local Alignment Search Tool of National Center for Biotechnology Information (Bethesda, Maryland, USA).

In Fig. 3, the similarity index analysis indicated that the bacterial communities of fried and boiled fish cakes during the storage could be divided into 2 clusters, their similarity ranging from 0.25 to 0.58. All the samples had band pattern similarity lower than 0.6 showing that the bacterial communities exhibited some change during the storage.

Tab. 1 shows all of the identified bands and the identified bacteria changing during the storage. The bacterial diversity of the boiled fish cakes was a little more complex than that of the fried fish cakes. *Pseudomonas poae* and Flavobacteriaceae dominated during entire storage time in the fried and boiled fish cakes (Tab. 2). The bacteria present were *Acinetobacter oleivorans*, *Riemerella anatipestifer*, *Staphylococcus aureus*, *Streptococcus aureus* and *Psychrobacter arcticus* in the middle of the shelf life of boiled fish cakes. In the middle of the shelf life of fried fish cakes, *Bacillus pumilus*, *Weissella koreensis* and *Psychrobacter* sp. were present. At the end of shelf life, *Psychrobacter* sp. was dominant in both samples (Tab. 2).

According to bulk-plate analysis and direct DNA approach, *Pseudomonas*, *Flavobacteriaceae* and *Psychrobacter* occured most frequently in fried and boiled samples during the storage period. This indicated that one or all of these bacteria greatly contributed to the overall decay of the samples during their storage. *Pseudomonas* spp. are reported to be the dominant spoilage bacteria of surimi-based imitation crab and kamaboko [19, 20]. These microorganisms were reported to correlate well with meat decay, characterized by the development of sweet, fruity off-odours in chilled fish [21, 22]. Imitation crab and kamaboko are the classic surimi products in Japan and European countries, like fish cake is the classic surimi product in China.

Flavobacteriaceae were common spoilers of cooked surimi-based cooked products and fresh

Tab. 2. Results after sequencing of the dominant bands from DGGE profiles.

Storage	Bulk cell DNA from PCA	Band No	Direct DNA from fish cakes	Band No
Boiled fish cakes				
0 days	*Pseudomonas synxantha* *Pseudomonas poae* Flavobacteriaceae	1, 2 3 4	*Psychrobacter* sp.	6, 7
1 day	*Pseudomonas poae* Flavobacteriaceae *Riemerella anatipestifer*	9 10 13	*Pseudomonas poae* Flavobacteriaceae	15 16
2 days	*Pseudomonas poae* Flavobacteriaceae *Streptococcus aureus* *Pseudomonas* sp.	15 16 18 19	Flavobacteriaceae *Pseudomonas poae*	16 21, 22
3 days	*Pseudomonas poae* Flavobacteriaceae	21 23	*Pseudomonas poae* Flavobacteriaceae *Psychrobacter* sp.	3 4 24
Fried fish cakes				
0 days	*Pseudomonas poae* Flavobacteriaceae *Pseudomonas poae* *Pseudomonas synxantha* *Pseudomonas moraviensis*	3 4 25 26 27	*Psychrobacter* sp. *Pseudomonas moraviensis*	24 30
3 days	*Pseudomonas poae* Flavobacteriaceae *Pseudomonas synxantha*	3 4 31	*Bacillus pumilus* *Weissella koreensis* *Pseudomonas moraviensis* *Psychrobacter* sp.	32 33 35 39
6 days	*Pseudomonas synxantha* *Pseudomonas poae* Flavobacteriaceae	36 37 41	*Pseudomonas poae* *Psychrobacter* sp. Flavobacteriaceae	37 39 41
8 days	*Pseudomonas poae* Flavobacteriaceae	40 41	*Pseudomonas poae* Flavobacteriaceae *Psychrobacter* sp.	40 41 42

Band numbers correspond to bands in Fig. 2 and Tab. 1. Only sequences with similarity > 97% are included in the table.

fish, though they were less competitive than other common spoilage organisms [23, 24]. The species of the genus *Psychrobacter*, which were only detected by culture-independent methods, were established spoilers of chilled high-protein food and were found in some kinds of surimi products, like kamaboko and chikuwa [25]. The work of BJØRKEVOLL et al. indicated that *Psychrobacter* sp. was responsible for the musty odour in the salt-cured cod [26]. Another study about ice storage ray also indicated that the co-dominating *Psychrobacter cryohalolentis*, *Pseudomonas* spp. and *P. cibarius* were able to produce spoilage volatile organic compounds [4]. Further research into the co-dominance of *Psychrobacter* spp. and *Pseudomonas* spp. on spoiled surimi would be useful to reveal their roles in the spoilage of fish cakes. *Weissella koreensis* was the other spoilage bacterium in the fried fish cakes. This bacterium was isolated as spoilage from kimchi, but was not found in meat or aquatic products before [27].

Some differences were observed between the microorganisms found by plate culture and PCR-DGGE methods, some microorganisms being found by the former method but not found by the latter. Previous study reported that the detection limit of DGGE ranged from 10^3 CFU·g^{-1} to 10^8 CFU·g^{-1} [8]. Thus the high and low density species could not be detected by PCR-DGGE. By the use of bulk plate DNA combined with DGGE, the low abundant live species could be detected in the DGGE profile. With the use of selective agar, the high plate counts of lactic acid bacteria could be found. These results indicated that the use of multiple methods could facilitate a more comprehensive analysis of microorganisms.

CONCLUSIONS

Based on culture-based and culture-independent methods, no significant difference was observed between the storage of fried and boiled fish cakes. *Weissella koreensis* was found, for the first time, in fish cakes. *Flavobacteriaceae*, *Pseudomonas poae* and *Psychrobacter* sp. were the dominant microbiota members in DGGE profiles. Plate counts on selective agar showed that the number of lactic acid bacteria were the highest. These are known spoilage organisms of meat products. Although it is not clear whether the microorganisms we detected in the DGGE profiles were live or not, it is clear that these microorganisms had lived at a certain stage of storage. Unfortunately, the direct DNA approach was unable to detect lactic acid bacteria, which would be worth to investigate in the future using a set of primers specific for this bacterial group [28].

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REFERENCES

1. Wang, X. – Hirata, T. – Fukuda, Y. – Kinoshita, M. – Sakaguchi, M.: Acceptability comparison of kamaboko gels derived from silver carp surimi and from walleye pollack surimi between the Chinese and Japanese. Fisheries Science, *68*, 2002, pp. 165–169. DOI: 10.1046/j.1444-2906.2002.00403.x.
2. Benjakul, S. – Visessanguan, W. – Thongkaew, C. – Tanaka, M.: Effect of frozen storage on chemical and gel-forming properties of fish commonly used for surimi production in Thailand. Food Hydrocolloids, *19*, 2005, pp. 197–207. DOI: 10.1016/j.foodhyd.2004.05.004.
3. GB10132 – 2005. Hygienic standard for aquatic products. Beijing : The ministry of health of the People's Republic of China, 2005. In Chinese.
4. Broekaert, K. – Heyndrickx, M. – Herman, L. – Devlieghere, F. – Vlaemynck, G.: Seafood quality analysis: molecular identification of dominant microbiota after ice storage on several general growth media. Food Microbiology, *28*, 2011, pp. 1162–1169. DOI: 10.1016/j.fm.2011.03.009.
5. Vogel, B. F. – Venkateswaran, K. – Satomi, M. – Gram, L.: Identification of *Shewanella baltica* as the most important H_2S-producing species during iced storage of Danish marine fish. Applied and Environmental Microbiology, *71*, 2005, pp. 6689–6697. DOI: 10.1128/AEM.71.11.6689-6697.2005.
6. Godálová, Z. – Kraková, L. – Puškárová, A. – Bučková, M. – Kuchta, T. – Piknová, Ľ. – Pangallo, D.: Bacterial consortia at different wine fermentation phases of two typical Central European grape varieties: Blaufränkisch (Frankovka modrá) and Grüner Veltliner (Veltlínske zelené). International Journal of Food Microbiology, *217*, 2016, pp. 110–116. DOI: 10.1016/j.ijfoodmicro.2015.10.015.
7. Šaková, N. – Sádecká, J. – Lejková, J. – Puškárová, A. – Koreňová, J. – Kolek, E. – Valík, Ľ. – Kuchta, T. – Pangallo, D.: Characterization of May bryndza cheese from various regions in Slovakia based on microbiological, molecular and principal volatile odorants examination. Journal of Food and Nutrition Research, *54*, 2015, pp. 239–251. issn: 1336-8672. <http://www.vup.sk/download.php?bulID=1657>
8. Ercolini, D.: PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. Journal of Microbiological Methods, *56*, 2004, pp. 297–314. DOI: 10.1016/j.mimet.2003.11.006.
9. Hovda, M. B. – Lunestad, B. T. – Sivertsvik, M. –

Rosnes, J. T.: Characterisation of the bacterial flora of modified atmosphere packaged farmed Atlantic cod (Gadus morhua) by PCR-DGGE of conserved 16S rRNA gene regions. International Journal of Food Microbiology, *117*, 2007, pp. 68–75. DOI: 10.1016/j.ijfoodmicro.2007.02.022.

10. Rudi, K. – Maugesten, T. – Hannevik, S. E. – Nissen, H.: Explorative multivariate analyses of 16S rRNA gene data from microbial communities in modified-atmosphere-packed salmon and coalfish. Applied and Environmental Microbiology, *70*, 2004, pp. 5010–5018. DOI: 10.1128/AEM.70.8.5010-5018.2004.
11. Jiang, Y. – Gao, F. – Xu, X. – Ye, K. – Zhou, G.: Changes in the composition of the bacterial flora on tray-packaged pork during chilled storage analyzed by PCR-DGGE and real-time PCR. Journal of Food Science, *76*, 2011, pp. M27–M33. DOI: 10.1111/j.1750-3841.2010.01879.x.
12. Jiang, Y. – Gao, F. – Xu, X. L. – Su, Y. – Ye, K. P. – Zhou, G. H.: Changes in the bacterial communities of vacuum-packaged pork during chilled storage analyzed by PCR-DGGE. Meat Science, *86*, 2010, pp. 889–895. DOI: 10.1016/j.meatsci.2010.05.021.
13. Sambrook, J. – Fritsch, E. F. – Maniatis, T.: Molecular cloning. A laboratory manual. 2nd ed. New York : Cold Spring Harbor Laboratory Press, 1989. ISBN: 0879693096.
14. Liu, Y. – Li, J. – Zhu, J. – Ma, Y. – Li, X.: Effects of modified atmosphere packaging on qualities of surimi product during refrigerated storage. Transactions of the Chinese Society of Agricultural Engineering, *26*, 2010, pp. 329–334. ISSN: 1002-6819. DOI: 10.3969/j.issn.1002-6819.2010.07.058. In Chinese.
15. Parlapani, F. F. – Meziti, A. – Kormas, K. A. – Boziaris, I. S.: Indigenous and spoilage microbiota of farmed sea bream stored in ice identified by phenotypic and 16S rRNA gene analysis. Food Microbiology, *33*, 2013, pp. 85–89. DOI: 10.1016/j.fm.2012.09.001.
16. Samelis, J. – Kakouri, A. – Georgiadou, K. – Metaxopoulos, J.: Evaluation of the extent and type of bacterial contamination at different stages of processing of cooked ham. Journal of Apply Microbiology, *84*, 1998, pp. 649–660. DOI: 10.1046/j.1365-2672.1998.00392.x.
17. Hinton, A., Jr. – Cason, J. A. – Ingram, K. D.: Tracking spoilage bacteria in commercial poultry processing and refrigerated storage of poultry carcasses. International Journal of Food Microbiology, *91*, 2004, pp. 155–165. DOI: 10.1016/S0168-1605(03)00377-5.
18. Samelis, J. – Kakouri, A. – Rementzis, J.: Selective effect of the product type and the packaging conditions on the species of lactic acid bacteria dominating the spoilage microbial association of cooked meats at 4 °C. Food Microbiology, *17*, 2000, pp. 329–340. DOI: 10.1006/fmic.1999.0316.
19. Fukao, T. – Ohyabu, S. – Sawada, H. – Ohta, Y.: Effects of addition of powdered protein to Kamaboko on growth of *Pseudomonas fluorescens*. Fisheries Science, *64*, 1998, pp. P57–P61. ISSN: 0919-9268. DOI: 10.2331/fishsci.64.57.
20. Yoon, I. H. – Matches, J. R. – Rasco, B.: Microbiological and Chemical Changes of Surimi-Based Imitation Crab during Storage. Journal of Food Science, *53*, 1988, pp. 1343–1346. DOI: 10.1111/j.1365-2621.1988.tb09273.x.
21. Reynisson, E. – Lauzon, H. L. – Magnússon, H. – Jónsdóttir, R. – Ólafsdóttir, G. – Marteinsson, V. – Hreggviðsson, G. O.: Bacterial composition and succession during storage of North-Atlantic cod (*Gadus morhua*) at superchilled temperatures. BMC Microbiology, *9*, 2009, pp. 250. DOI: 10.1186/1471-2180-9-250.
22. Olafsdottir, G. – Lauzon, H. L. – Martinsdottir, E. – Kristbergsson, K.: Influence of storage temperature on microbial spoilage characteristics of haddock fillets (*Melanogrammus aeglefinus*) evaluated by multivariate quality prediction. International Journal of Food Microbiology, *111*, 2006, pp. 112–125. DOI: 10.1016/j.ijfoodmicro.2006.04.045.
23. Masniyom, P.: Deterioration and shelf-life extension of fish and fishery products by modified atmosphere packaging. Sonklanakarin Journal of Science and Technology, *33*, 2011, pp. 181–192. ISSN: 0125-3395. <http://rdo.psu.ac.th/sjstweb/journal/33-2/0125-3395-33-2-181-192.pdf>
24. Ingham, S. C. – Potter, N. N.: Microbial growth in surimi and mince made from Atlantic pollock. Journal of Food Protection, *50*, 1987, pp. 312–315. ISSN: 0362-028X (print), 1944-9097 (online).
25. Tsuda, K. – Nagano, H. – Ando, A. – Shima, J. – Ogawa, J.: Isolation and characterization of psychrotolerant endospore-forming *Sporosarcina* species associated with minced fish meat (surimi). t Journal of Food Microbiology, *199*, 2015, pp. 15–22. DOI: 10.1016/j.ijfoodmicro.2014.12.031.
26. Bjorkevoll, I. – Olsen, R. L. – Skjerdal, O. T.: Origin and spoilage potential of the microbiota dominating genus *Psychrobacter* in sterile rehydrated salt-cured and dried salt-cured cod (*Gadus morhua*). International Journal of Food Microbiology, *84*, 2003, pp. 175–187. DOI: 10.1016/S0168-1605(02)00418-X.
27. Choi, H. – Kim, Y. W. – Hwang, I. – Kim, J. – Yoon, S.: Evaluation of *Leuconostoc citreum* HO12 and *Weissella koreensis* HO20 isolated from kimchi as a starter culture for whole wheat sourdough. Food Chemistry, *134*, 2012, pp. 2208–2216. DOI: 10.1016/j.foodchem.2012.04.047.
28. Sádecká, J. – Šaková, N. – Pangallo, D. – Koreňová, J. – Kolek, E. – Puškárová, A. – Bučková, M. – Valík, Ľ. – Kuchta, T.: Microbial diversity and volatile odour-active compounds of barrelled ewes' cheese as an intermediate product that determines the quality of winter bryndza cheese. LWT – Food Science and Technology, *70*, 2016, pp. 237–244. DOI: 10.1016/j.lwt.2016.02.048.

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