

Bacterial Diversity, Biogenic Amines and Lipids Oxidation in Traditional Dried Anchovy (*Encrasicholina punctifer*) during Ambient Storage

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Abstract

This study aimed to elucidate the effect of ambient storage ($23\pm 2^{\circ}\text{C}$, 68% RH) on the bacterial load and diversity, biogenic amines and lipids oxidation in traditional dried anchovy (*E. punctifer*) in order to evaluate its safety, quality and stability during 12 weeks of storage. Total aerobic bacteria (TAB), *Staphylococcus aureus*, *Enterobacteriaceae* (ENT), histidine decarboxylating bacteria (HDB), lysine decarboxylating bacteria (LDB) and ornithine decarboxylating bacteria (ODB) were enumerated and identified by conventional, VITEK 2 compact and sequencing of 16S rRNA gene methods. Histamine, cadaverine and putrescine contents were determined by high performance liquid chromatography. Lipid oxidation was evaluated by peroxide value (PV). Total aerobic bacteria, *S. aureus*, ENT, HDB, LDB and ODB initial counts of \log_{10} 4.9 ± 0.85 , 3.7 ± 0.57 , 4.2 ± 0.05 , 3.7 ± 0.72 , 3.9 ± 0.40 and 4.1 ± 0.24 CFU/g respectively did not significantly change ($p > 0.05$) during 12 weeks of storage. A high bacterial diversity of 27 species belonging to 20 genera was found, with the dominance of *S. aureus*, *Acinetobacter lwoffii* and *S. warneri* and the first incidence of *Psychrobacter celer*, *Desemzia incerta*, *Granulicatella elegans* and *Bhargavaea indica* in dried fish. Initial histamine, cadaverine and putrescine contents and PV of 5.2 ± 4.3 , 8.5 ± 1.9 and 5.8 ± 0.6 mg/100g and 0.19 ± 0.02 meq/kg respectively did not significantly change ($p > 0.05$) during 12 weeks of storage. This study found that ambient storage at $23\pm 2^{\circ}\text{C}$, 68% RH for 12 weeks did not affect the bacterial load, biogenic amines and lipids, and that the dried anchovy remained microbiologically safe and of good quality.

Keywords: Traditional anchovy; Diversity; Ambient; Biogenic amines

1 Introduction

Traditionally, fresh anchovy is caught by trawler fishing, handled under unhygienic conditions and

kept at ambient temperature for several hours, spread on sandy/clay sites and sun-dried for 3–5 days in open coastal areas. Pre-drying treatments such as washing and salting are not con-

Nomenclature

TAB	total aerobic bacteria	LDB	lysine decarboxylating bacteria
ENT	<i>Enterobacteriaceae</i>	ODB	ornithine decarboxylating bacteria
HDB	histidine decarboxylating bacteria	PV	peroxide value

ducted in some developing countries such as Oman.

Salting, fermenting, drying and smoking are the common fish preservation methods used in the developing countries, particularly Africa and Asia, where these methods represented 12 percent of all fish destined for human consumption in the developing countries in 2016 (Food and Agriculture Organization, 2018).

These practices are expected to increase bacterial load and diversity and to heavily expose fish products to contamination with pathogenic, spoilage and biogenic amine producing bacteria from different sources during catching and prior to and post processing. However, this diversity is not well understood in dried fish products such as dried anchovy. In a single study, *S. aureus* was found in some dried fish products (Moon, Min, Park, Park & Yoon, 2017).

Moreover, since fish is exposed to different contamination sources in traditional drying and dried fish products are stored at ambient temperature in many developing countries, high bacterial diversity in dried fish is expected as a result of these conditions. Nevertheless, this diversity has not been explored and elucidated in these products in order to assess their microbial safety and quality.

Histamine levels higher than 5 mg/100g were found in some dried fish such as dried flying fish (Kung et al., 2015). In addition, histamine-producing bacteria such as *Raoultella ornithinolytica*, *Pantoea agglomerans*, *Proteus vulgaris* and *Enterobacter amnigenus* isolated from dried mahi-mahi were found to be capable of producing 1.25-56.2 mg/100 g of histamine (Lin et al., 2014). Besides their association in scombroid

food poisoning, biogenic amines can be used to evaluate the hygienic handling and quality of fish. In this regard, histamine can be used to evaluate the quality of dark muscled fish, whereas putrescine and cadaverine are more subjective parameters to evaluate the quality of white muscled fish and other seafood products (Prester, 2011). Moreover, cadaverine and putrescine have been shown to be involved in the formation of nitrosamines, nitrosopiperidine (NPIP), and nitrosopyrrolidine (NPYR), respectively in in-vitro studies and factors such as impure salts and high temperature have been found to enhance nitrosamine formation (Al Bulushi, Poole, Deeth & Dykes, 2009). Since dried fish are stored at ambient temperature for months before consumption, the stability of biogenic amines during storage needs to be well understood in order to ensure the safety of these products.

Fresh anchovy contains $12.79 \pm 0.53\%$ lipid (Gencbay & Turhan, 2016). Fish with this lipids' content is subject to lipid oxidation during processing and storage. Peroxide value is used as a main parameter to assess lipid oxidation (Milijašević, Babić Milijašević, Đinović-Stojanović, Vesković Moračanin & Slobodan, 2017). Information on the lipid oxidation in dried fish processed and stored traditionally which is required to evaluate the quality and storage stability of the products is also limited.

Therefore, this study aimed to elucidate the effect of ambient storage ($23 \pm 2^\circ\text{C}$, 68% RH) on bacterial load and diversity, content of biogenic amines and lipid oxidation in order to evaluate the safety, quality and storage stability of tradi-

tional dried anchovy.

2 Materials and Methods

2.1 Dried anchovies and storage conditions

Fresh anchovies were caught from the Sea of Oman and the whole ungutted anchovies were dried by spreading on sandy/clay open sites under the sun for 3-5 days at a temperature of approximately 25°C - 35°C. In Arabian Gulf countries such as Oman and UAE, fish are not salted prior to drying. Moreover in traditional practices, the end point of drying, the quality and safety of fresh anchovies are not evaluated. Two storage studies were conducted. For each storage study about 15 kg of dried anchovy (*Encrasicholina punctifer*) was purchased from a local processing site in UAE immediately after drying. Dried anchovies were dispensed into 500 g samples in closed polyethylene bags and stored at ambient temperature (23±2°C, 68% RH) for 12 weeks of storage. This temperature was selected because 23±2°C is a common storage temperature for this product in many developing counties. Samples were analyzed at three-week intervals over 12 weeks. At each sampling occasion, three samples of dried anchovies, each weighing 500 g, were used for analyses. Prior to analyses, dried anchovies were aseptically chopped manually and mixed. The same chopped sample was used for all analyses.

2.2 Bacterial enumeration and identification

Total aerobic bacteria were enumerated on tryptone soya agar (TSA) (Oxoid, UK), supplemented with 2% NaCl (Oxoid, UK) and incubated aerobically at 32 °C for three days (Al Bulushi, Poole, Deeth & Dykes, 2008). To recover the injured bacteria of specific groups, a thin agar layer method (TAL) was used in the selective media (Wu, 2008). In this method, Baird-Parker agar and ISO violet red bile glucose agar were overlaid with 6 mL of TSA before inoculation. *Staphylococcus aureus* was enumerated on Baird-Parker agar (Oxoid, UK),

supplemented with rabbit plasma fibrinogen (Oxoid, UK) and incubated at 37°C for 48 ± 2 h (Reginald & Gayle, 2016). *Staphylococcus aureus* production of coagulase as a means of identification was confirmed by the Staphytest plus system (Oxoid, UK). *Enterobacteriaceae* were enumerated on ISO violet red bile glucose agar (VRBG, Oxoid, UK) and incubated at 37°C for 24 ± 2 h (ISO, 2004). Histidine decarboxylating bacteria, LDB and ODB were enumerated on HD-medium and plates were incubated aerobically at 30 °C for two and four days (Yamani & Untermann, 1985). This medium consisted of 0.5% tryptone (Oxoid, UK), 0.5% yeast extract (Oxoid, UK), 2.7% L-histidine.2HCl (Sigma, Germany), 0.5% NaCl (Oxoid, UK), 0.1% CaCO₃ (Sigma, Germany), 2% agar (Oxoid, UK) and 0.006% bromocresol purple (Sigma, Germany), and the pH was adjusted to pH 5.3. For enumeration of LDB and ODB, L-histidine.2HCl was replaced by L-lysine monohydrochloride (Sigma, Germany) and L-ornithine monohydrochloride (Sigma, Germany) respectively. To facilitate the enumeration of LDB and ODB, 0.01% pyridoxine hydrochloride (Sigma, USA) was added as a coenzyme (Frank, Baranowski, Chongsiriwatana, Brust & Premaratne, 1985).

Eighteen isolates were randomly selected using Harrison's disc for randomized colony selection (Harrison 1938, cited by Harrigan, 1998) at each sampling occasion. The isolates were purified twice in TSA, supplemented with 2% agar and stored on beads (Abtek, UK) at -80 °C until identified. Isolates were identified to the genus and species levels by VITEK 2 compact (bioMérieux, France), using GP and GN cards and software version of 05.02 according to the manufacturer's instructions, and by sequencing of the 16S rRNA gene. The 16S rRNA gene of selected strains was amplified by the PCR procedure described by Ayyash et al. (2018). PCR primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') were employed during amplification. PCR mixtures were prepared following the manufacturer's protocol (Qiagen, Cat No./ID: 201443) and subjected to initial denaturation at 94°C for 2 min followed by 35 cycles of

heating at 94°C (20 sec), primer annealing at 53°C (20 sec) and extension at 70°C for 1.5 min. The final extension was carried out at 70°C for 5 min for 1 cycle. Presence of specific PCR products was confirmed by agarose electrophoresis. The DNA sequence of PCR products was carried out by MacroGen Sequencing Facilities (<http://dna.macrogen.com>, Seoul, Korea). Sequence results were aligned with the NCBI database using the BLAST algorithm. BLAST and probabilities were carried out by MacroGendna.com. A probability level of $\geq 93\%$ was considered for bacterial identification by VITEK 2 compact.

2.3 Determination of water content, water activity, peroxide value and color

Water content was determined gravimetrically by drying at 105°C in an air convection drier (Gallenkamp, UK) to a consistent weight (Association of Official Analytical Chemists, 1990). Water activity was measured by a water activity meter (Rotronic, USA). Peroxide value was measured using a method provided by Egan, Kirk and Sawyer (1981). Briefly, 0.5 g of dried anchovies was mixed with 25 mL of a mixed solution (acetic acid (Sigma, Germany): chloroform (Sigma, Germany), 3:2). Then, 1 mL of saturated potassium iodide (Sigma, Germany) was added and the sample was kept in a dark place for 10 min. A total of 30 mL of water was added and the liberated iodine was titrated with 0.01 N sodium thiosulfate (Sigma, Germany) in the presence of 1 mL of freshly prepared 1% starch (Sigma, Germany) until the disappearance of the blue color. Peroxide value was calculated as meq/kg lipid according to the following formula:

$$PV = (A - B)/S \times 10 \quad (1)$$

Where, A is the titration value for the sample, B is the titration value for the blank, and S is the weight of the sample.

The color of dried anchovy was measured using a color meter, Minolta Chroma meter (Model CR-310, Japan) and a method followed by Rahman, Al-Amri and Al-Bulushi (2002). Briefly,

the equipment was calibrated with a white standard calibration plate provided by the manufacturer. Six dried anchovies were placed on a flat surface, the tip of the measuring head was pointed on the sample and the color measurement was taken. Five readings for each value from each sample were recorded. The results were expressed in Hunter as L, a and b values, where L is lightness or darkness (black L = 0; white L = 100), a is intensity of red color and b is intensity of yellow color.

2.4 Determination of amino acids decarboxylation activity

The abilities of the isolates to decarboxylate histidine, lysine and ornithine were assessed using HD-medium developed by Yamani and Untermann (1985). This medium was composed of 2 g peptone, 1 g Lab-lemco powder, 5 g NaCl, 10 g L-histidine monohydrochloride monohydrate (Sigma, Germany), 10 mL bromo-cresol green solution 0.1%, 10 mL chlorophenol red solution 0.2% and 1000 mL deionized water. Pyridoxine hydrochloride was added to the medium to facilitate the decarboxylation of lysine and ornithine. Briefly, 100 μ L aliquot of 24 h old isolates was inoculated in HD-medium; the medium was immediately sealed with mineral oil and incubated at 32 °C for 4 days. The presence of amino acid decarboxylase was assessed by changing the color of the medium from green to violet.

2.5 Determination of biogenic amines

Whole dried anchovy was ground using a commercial blender (Black and Decker, USA). Ground dried anchovies (5 g) were homogenized for 2 min at high speed in a homogenizer (Black and Decker, USA) with 20 mL chilled 6% trichloroacetic (TCA) (Sigma, USA) in a 50-mL centrifuge tube for 3 min. The homogenates were centrifuged at 10,000 g for 10 min at 4 °C and filtered through Whatman No. 2 filter paper (Sigma, USA). The filtrates were transferred in a 50-mL volumetric flask and brought to a final volume of 50 mL with TCA. Aliquots (20 mL) were placed into storage vials and stored at -50

°C until use. Histamine, cadaverine and putrescine contents were determined by HPLC using a Lichrospher100 RP-18 reversed-phase column (5 μ m, 125 \times 4.6 mm, E. Merck, Darmstadt, Germany). The mobile phase consisted of water and methanol (Sigma, Germany). The gradient elution program was started with 50/50 (methanol : water) at a low rate of 0.8 mL/min for 5 min. Then the gradient elution was followed by a linear increase to 85/15 (methanol : water) at the same flow rate for 6.5 min. The latter protocol was held for another 5 min and then decreased to 50/50 (0.8 mL/min) for the last 2 min.

The standards consisted of putrescine dihydrochloride (Put), cadaverine dihydrochloride (Cad) and histamine dihydrochloride (Him) (Sigma, Germany). Put (91.5 mg), Cad (85.7 mg) and Him (82.8 mg) were prepared in 50 mL of 0.1 M HCl and used as the standard stock solution (each at 1.0 mg/mL). Before injection to HPLC, the sample and standards were derivatized and the biogenic amines contents were determined as described by Tsai et al. (2005).

2.6 Statistical analysis

Bacterial numbers are reported as log₁₀ CFU/g. A one-way ANOVA test was used to evaluate the effect of ambient storage on the parameters, whereas Tukey Simultaneous Test was used to evaluate the differences between the initial and final values of each parameter during storage. These tests were conducted in Minitab release 14 software (Minitab Inc., USA), and a level $p < 0.05$ was considered statistically significant. Each sample was run in 2-6 replicates.

3 Results and Discussion

3.1 Bacterial counts

Changes in bacterial counts during ambient storage can be seen in Table 1. The total aerobic bacterial count of log 4.9 ± 0.85 CFU/g indicates the good microbial quality of dried anchovies as compared with log 5 CFU/g set for good quality foods (International Commission on Microbiological Specifications for Foods, 1986). This count may also indicate the dominance of meso-

philic bacteria in dried anchovies during ambient storage as anchovies are heavily contaminated during handling at ambient temperature. In fact, the dominance of mesophilic bacteria in dried anchovies prior and during ambient storage should be expected since dried anchovies are handled at ambient temperature and this group grow at ambient temperature with an optimum temperature of 35°C (Ray & Bhunia, 2014). It is expected that the main sources of mesophilic bacteria in dried anchovies are drying surfaces, air born bacteria, human contact and packaging. The practices of traditional catching such as exposure to poor hygienic conditions during catching, handling and drying were expected to heavily increase TAB in dried anchovies, however, the TAB were found to be within the limit of good quality food (International Commission on Microbiological Specifications for Foods, 1986).

It is quite possible that traditional drying times e.g. 5 days and direct sunlight either killed some bacteria or caused cell injury to others. Moreover, the water activity of about 0.5 (Table 2) was another inhibitory factor to limit bacterial growth to log 4.9 ± 0.85 CFU/g. Total aerobic bacterial count in our study was lower than the log 8 CFU/g found in some dried fish (Jakhar, Kumar & Vardia, 2015). This discrepancy may reflect the effect of fish environment and hygienic handling status on the bacterial load of dried fish. Anchovies which were used in this study were harvested from the sea whereas those used in that study (Jakhar et al., 2015) were harvested from fresh water. These environments have different microbial flora which can be a source of microbial flora in fish besides handling (Al Bulushi et al., 2009). Total aerobic bacteria counts did not significantly change ($p > 0.05$) during ambient storage for 12 weeks. This trend can be expected as water activity (Table 2) did not exceed 0.5 during storage which is an inhibitory value for bacterial growth.

Dried anchovies were initially loaded with log 3.7 ± 0.57 CFU/g and 4.2 ± 0.05 CFU/g of *S. aureus* and *Enterobacteriaceae* respectively. The *S. aureus* count in the current study was higher than that found in some dried fish (Kakati, Sharma & Goswami, 2017). However, this count is not expected to create any safety risk factor as a typical count of 10^5 -

Table 1: Bacterial counts in dried anchovies during storage at 23±2°C, 68% RH

Time, week	Bacterial count, log ₁₀ CFU/g					
	TAB	<i>S. aureus</i>	ENT	HDB	LDB	ODB
0	4.9 ± 0.85 ^a	3.7 ± 0.57 ^a	4.2 ± 0.05 ^a	3.7 ± 0.72 ^a	3.9 ± 0.40 ^a	4.1 ± 0.24 ^a
3	4.5 ± 0.80 ^a	3.5 ± 0.34 ^a	3.1 ± 1.1 ^a	2.7 ± 0.73 ^a	3.7 ± 0.31 ^a	3.5 ± 0.02 ^a
6	4.6 ± 0.92 ^a	3.9 ± 0.27 ^a	3.2 ± 0.49 ^a	3.0 ± 0.22 ^a	4.2 ± 0.40 ^a	4.0 ± 0.85 ^a
9	4.1 ± 1.5 ^a	3.7 ± 0.23 ^a	3.3 ± 0.49 ^a	3.8 ± 0.65 ^a	4.7 ± 0.04 ^a	4.7 ± 0.15 ^a
12	4.8 ± 0.51 ^a	4.0 ± 0.04 ^a	3.2 ± 0.16 ^a	3.8 ± 0.38 ^a	3.9 ± 0.69 ^a	4.0 ± 0.40 ^a

Each mean was compared with that at week 0. Means with different alphabetical superscripts in the same column are significantly different ($p < 0.05$), $n = 3$

TAB: total aerobic bacteria, EN: *Enterobacteriaceae*, HDB: histidine decarboxylating
LDB: lysine decarboxylating bacteria, ODB: ornithine decarboxylating bacteria

Table 2: Water content, water activity and biogenic amines contents in dried anchovies during storage at 23±2°C, 68% RH

Time, week	% water	a_w	PV, meq/kg	His, mg/kg	Cad, mg/kg	Put, mg/kg
0	8.3 ± 0.03 ^a	0.48 ± 0.00 ^a	0.19 ± 0.02 ^a	5.252 ± 4.3 ^a	8.585 ± 1.9 ^a	5.858 ± 0.6 ^a
3	8.3 ± 0.04 ^a	0.48 ± 0.00 ^a	0.34 ± 0.15 ^a	8.282 ± 5.5 ^a	5.656 ± 0.75 ^a	5.252 ± 0.5 ^a
6	8.5 ± 0.43 ^a	0.49 ± 0.00 ^b	0.28 ± 0.12 ^a	3.838 ± 2.6 ^a	7.676 ± 4.0 ^a	9.090 ± 3.5 ^a
9	7.9 ± 0.34 ^a	0.50 ± 0.00 ^b	0.20 ± 0.01 ^a	2.424 ± 0.84 ^a	6.666 ± 1.3 ^a	5.656 ± 1.3 ^a
12	9.1 ± 0.14 ^b	0.51 ± 0.00 ^b	0.30 ± 0.11 ^a	5.656 ± 3.8 ^a	3.838 ± 1.0 ^a	3.838 ± 00 ^a

Each mean was compared that at week 0. Means with different alphabetical superscripts in the same column are significantly different ($p < 0.05$), $n = 2-6$

PV: peroxide value, His: histamine, Cad: cadaverine, Put: putrescine

10⁸ CFU/g of *S. aureus* is required for enterotoxin production and secondly, the enterotoxins production requires a water activity level of 0.85-1.0 which was not provided by dried anchovies in the current study (Montville & Matthews, 2008; Stewart, 2003). The *Enterobacteriaceae* load indicated that the product had been exposed to cross-contamination of sanitary sources, especially from feces of animals. In fact, as per traditional processing, anchovies are processed in open sites where different animals have access to the products during processing. Although most *Enterobacteriaceae* are heat-sensitive (Baylis, Uyttendaele, Joosten & Davies, 2011), the presence of these microorganisms in anchovies indicates that traditional

drying temperature and time did not destroy *Enterobacteriaceae* totally and dried anchovies served as a vehicle for this group of microorganisms. The common *Enterobacteriaceae* sanitary sourced pathogens such as *Salmonella* sp. and *E. coli* which are expected to be among the flora due to contamination from humans during handling were not found among the *Enterobacteriaceae* in the current study. This absence may be attributable to the effect of the drying temperature. In fact, the counts of some *Enterobacteriaceae*, such as *Salmonella* sp. and *Salmonella typhimurium*, were found to decrease during drying (Ingham, Searls & Buege, 2006).

During ambient storage for 12 weeks, neither *S. aureus* nor *Enterobacteriaceae* showed statistic-

Table 3: Bacterial diversity in dried anchovies during storage at 23±2°C, 68% RH

Bacteria	AC/Bio #	Storage, week					#
		0	3	6	9	12	
<i>Macrococcus sp.</i>	KP181835.1	1					1
<i>Psychrobacter sp.</i>	FJ984919.1	2	1	1	2		6
<i>Staphylococcus sciuri</i>	KT955004.1	3				4	7
<i>Staphylococcus aureus*</i>		13	10	15	11	5	54
<i>Pseudomonas fluorescens</i>	5000001100101240	3					3
<i>Alloiococcus otitis</i>	000002000000000	5	6		3	10	24
<i>Aeromonas salmonicida</i>	0000000000000200	2				1	3
<i>Kocurica kristinae</i>	04000203220031	10	1	1		2	14
<i>Acinetobacter lwoffii</i>	0000000100000000	5	15	11	3	10	44
<i>Serratia fonticola</i>	616363563561101	1					1
<i>Staphylococcus xylosum</i>	430046057773231	6	4				10
<i>Micrococcus luteus</i>	041032301000000	1					1
<i>Staphylococcus warneri</i>	050002003220231	2	5	1	10	19	37
<i>Streptococcus alactolyticus</i>	000030310270021	1					1
<i>Staphylococcus saprophyticus</i>	050002012670231	1				1	2
<i>Sphingomonas paucimobilis</i>	0001200150300210	1	4			3	8
<i>Staphylococcus hominis</i>	000000000320231		2		1		3
<i>Staphylococcus gallinarum</i>	430446056373331		1				1
<i>Comamonas testosteroni</i>	0000000100500001		1				1
<i>Aerococcus viridans</i>	020103000042031		1				1
<i>Staphylococcus epidermidis</i>	KP236244.1		1	1			2
<i>Arthrobacter sp.</i>	JX047437.1		1			1	2
<i>Psychrobacter celer</i>	KR051247.1			5		1	6
<i>Pseudomonas oryzae</i>	4 001600140100210			1			1
<i>Kocuria rosea</i>	010010300000000				1	2	3
<i>Staphylococcus caprae</i>	010002002461221				1		1
<i>Sporosarcina aquimarina</i>	KT922020.1				1		1
<i>Desemzia incerta</i>	LN867201.1					1	1
<i>Methylobacterium sp.</i>	0000000200000000					1	1
<i>Granulicatella elegans</i>	010030300000000					1	1
<i>Pantoea sp.</i>	AY659872.1					1	1
<i>Bhargavaea indica</i>	KT008289.1					1	1
Total							243

AC : Accession number for sequencing of 16S rRNA gene

BIO : Bionumber in VITEK 2 compact

*: Identified by Staphylect plus system

ally significant change ($p > 0.05$). This trend was attributed to the low water activity of the product of around 0.5 whereas the minimum water activities required for the growth of *S. aureus* and *Enterobacteriaceae* are 0.8 and 0.94 respectively (Baylis et al., 2011). *S. aureus* viability in the current study agreed with Moon et al. (2017) who found a significant reduction in *S. aureus* only after 5 months of storage at 24°C.

The counts of HDB, LDB and ODB ranged from $\log 3.7 \pm 0.72$ CFU/g, $\log 3.9 \pm 0.40$ CFU/g and $\log 4.1 \pm 0.24$ CFU/g at the beginning of storage to $\log 3.8 \pm 0.38$ CFU/g, $\log 3.9 \pm 0.69$ CFU/g and $\log 4.0 \pm 0.40$ CFU/g at the end of storage respectively; these changes were not statistically significant ($p > 0.05$). The counts of HDB, LDB and ODB may indicate the potential of these flora to produce histamine, cadaverine and putrescine in fresh anchovies prior to drying.

3.2 Bacterial diversity

In total, 243 isolates were identified (Table 3). Twenty-seven species belonging to 20 genera were found, where the *Staphylococcus* genus was dominant with 49% of total bacteria, followed by the *Acinetobacter* genus with 18% of total bacteria. Throughout the storage, *S. aureus*, *Acinetobacter lwoffii* and *S. warneri* dominated the bacterial flora with 22%, 18% and 15% of total bacteria respectively. *S. aureus* was the dominant species within the *Staphylococcus* genus with 45% followed by *S. warneri* with 31%. Most of bacteria maintained their viability during ambient storage, indicating high diversity of bacteria in dried anchovy. In general, the incidences of Gram-positive bacteria in dried anchovy were higher than those of Gram-negative bacteria. To our knowledge, this study is the first to report the incidences of some bacteria such as *Psychrobacter celer*, *Desemzia incerta*, *Granulicatella elegans* and *Bhargavaea indica* in dried fish. High bacterial diversity and incidences in dried anchovies could be attributed to high exposure to cross-contamination, mainly from sand and humans during handling and processing. In fact, high *S. aureus* and *Alloiococcus otitis* incidence indicates high cross-contamination from hu-

mans (El-Jakee et al., 2008). Incidence and viability of *S. aureus* in dried anchovy in the current study coincided with that found in some dried fish (Moon et al., 2017).

Maintaining viability in dried anchovy confirmed the earlier finding that *S. aureus* resists drying (Beardpegler, Stubbs & Vickery, 1988). *S. aureus* enterotoxins have not been assessed in the current study, however, their presence in dried fish has not been reported in other studies to our knowledge. *S. warneri*, found at high levels in the current study has been reported in various marine fish (Musharrafiéh, Tacchi, Trujeque, LaPatra & Salinas, 2014). Its viability during the storage of dried product indicates its resistance to drying conditions. Among the Gram-negative bacteria, *A. lwoffii* dominated the flora. This infectious bacterium, which originates from humans, was found to resist the drying conditions; this could explain its viability and dominance in the current study (Jawad, Heritage, Snelling, GascoyneBinzi & Hawkey, 1996).

Among biogenic amines producing flora, HDB mainly dominated the flora by 15 % followed by LDB and ODB by 9 % (each) (Table 4). *S. warneri* showed the highest incidence of decarboxylation of histidine followed by decarboxylation of lysine and ornithine. *S. warneri* strains' abilities to decarboxylate histidine, lysine and ornithine in the current study agreed with Marino, Frigo, Bartolomeoli and Maifreni (2011) who found that 9 of 14 *S. warneri* strains decarboxylated histidine, lysine and ornithine. In fact, *Staphylococcus* sp. have been widely shown to have amino acid decarboxylation activities, mainly of histidine, lysine, ornithine and tyrosine; the main *Staphylococcus* sp. which showed decarboxylation activity of these amino acids include *S. xylosum*, *S. pasteurii*, *S. aureus*, *S. sciuri*, *S. warneri*, and *S. vitulinus* (Marino et al., 2011). Despite this potential, *Staphylococcus* sp. were found to be weaker formers of biogenic amines than certain Gram-negative species such as *Morganella morganii* (Rodríguez-Jerez, Mora-Ventura, López-Sabater & Hernández-Herrero, 1994). Moreover, the reasonably low biogenic amine contents in the current study could indicate that these biogenic amines had been formed mainly by weaker producers of biogenic amines such as *Staphylococcus* sp.

Table 4: Amino acid decarboxylation potentials of the bacterial flora of dried anchovies

Bacteria	HD	LD	OD
<i>Staphylococcus xylosus</i> (10)	6		2
<i>Micrococcus luteus</i> (1)	1		
<i>Staphylococcus warneri</i> (37)	15	2	1
<i>Streptococcus alactolyticus</i> (1)	1		
<i>Staphylococcus saprophyticus</i> (1)	1		1
<i>Kocurica kristinae</i> (14)	3	2	2
<i>Sphingomonas paucimobilis</i> (8)	6	1	
<i>Staphylococcus caprae</i> (1)	1		
<i>Staphylococcus hominis</i> (3)	1		
<i>Desemzia incerta</i> (1)	1		
<i>Sporosarcina aquimarina</i> (1)	1		
<i>Alloiococcus otitis</i> (24)		7	11
<i>Acinetobacter lwoffii</i> (44)		5	3
<i>Arthrobacter sp</i> (2)		1	
<i>Psychrobacter celer</i> (6)		1	1
<i>Psychrobacter sp.</i> (6)		1	
<i>Bhargavaea indica</i> (1)		1	
<i>Aeromonas salmonicida</i> (3)		1	
<i>Pseudomonas oryzihabitans</i> (1)			1
Total	37	22	22

HD: histidine decarboxylation;
LD: lysine decarboxylation;
OD: ornithine decarboxylation

Table 5: Color values in dried anchovies during storage at 23±2°C, 68% RH

Time, week	L value	a value	b value
0	47.2 ± 1.2 ^a	0.90 ± 0.11 ^a	6.2 ± 0.25 ^a
3	55.2 ± 2.8 ^b	1.1 ± 0.77 ^a	6.9 ± 0.22 ^a
6	39.0 ± 0.60 ^b	1.0 ± 0.19 ^a	6.5 ± 0.27 ^a
9	37.4 ± 0.50 ^b	0.88 ± 0.24 ^a	6.6 ± 0.46 ^a
12	35.7 ± 1.9 ^b	1.2 ± 0.49 ^a	6.6 ± 0.24 ^a

Each mean was compared with that of 0 week.

Means with different alphabetical superscripts in the same column are significantly different ($p < 0.05$), $n = 6$

3.3 Lipid changes

Peroxide value (PV), an indicator of lipid oxidation, was 0.19 ± 0.02 meq/kg at the beginning of storage and it did not significantly increase ($p > 0.05$) to 0.30 ± 0.11 meq/kg during ambient storage for 12 weeks. Peroxide values in the current study are not expected to induce any rancidity which is only noticeable at a PV of more than 10 meq/kg (Egan et al., 1981). Anchovy is a pelagic fatty fish which could be subjected to lipid oxidation, however, the low PV value in the current study indicated good dried anchovy's stability during 12 weeks of storage. The low PV value in the current study could be attributed to the effect of the high direct sun-drying temperature and ambient storage. The effect of processing temperature on the stability of lipids was reported by Ortiz et al. (2013) who found that drying at 60°C resulted in the formation of more lipid oxidation products than drying at 40°C. Whereas, the effect of ambient storage temperature was studied by Takiguchi (1996) who found that pulverized niboshi (boiled and dried anchovy) showed a decrease in PV during storage at 25°C for 60 days compared with storage at -20°C.

The PV value of the dried anchovy in the current study was lower than that found in some dried fish which might be explained by effects of some factors such as fish species, handling and conditions of traditional drying (Kakati et al., 2017). Lipids' stability in dried stored anchovies in the current study agreed with that found in *Stolephorus commersonnii* which was handled, dried and stored at similar conditions of dried anchovies (Patterson, Kailasam, Giftson & Immaculate, 2018). The color value of b (yellowness) has been found to increase with lipid oxidation via the interaction of oxidized products with amines in proteins (Thanonkaew, Benjakul, Visessanguan & Decker, 2006). In the current study, however, neither PV nor b value showed a significant change (Table 5).

3.4 Biogenic amines changes

The contents of histamine, cadaverine and putrescine in dried anchovies (Table 2) ranged from

52 ± 4.3 , 85 ± 1.9 and 58 ± 0.6 mg/kg at the beginning of storage to 56 ± 3.8 , 38 ± 1.0 and 38 ± 0 mg/kg at the end of storage; these changes were statistically non-significant ($p > 0.05$). In general, histamine levels in dried anchovy in our study did not exceed the FAO/WHO allowed limit for histamine of 200 mg/kg (Food and Agriculture Organization, 2012).

The contents of all biogenic amines in dried anchovy in the current study were found to be lower than those found in dried fish products such as flying fish, mahi-mahi and anchovy (Kung et al., 2015; Lin et al., 2014). This discrepancy may be attributable to different pre-drying conditions such as handling temperature and hygiene, to the different abilities of contaminating microbial flora to form biogenic amines and to different post-drying properties of the product such as water activity. The effects of these factors have been clearly elucidated in many studies (Kung et al., 2015; Lin et al., 2014). For instance, histamine reached 50 ppm in more than 12 h during on-board handling of mahi-mahi at 26°C, whereas, this level was attained within 9 h at 35°C (Staruszkiewicz et al., 2004). Certain bacteria such as *Enterobacter aerogenes* were found to produce more than 500 ppm histamine in trypticase soy broth supplemented with 1.0% L-histidine (Kung et al., 2015). All biogenic amines were stable during the 12-week ambient storage. This stability could be expected as the biogenic amine producers were inactive in the current study due to low water activity in dried anchovies. The stability of the biogenic amines in the current study is in agreement with that reported by Hwang et al. (2012).

4 Conclusions

This is the first study to show a high bacterial diversity in a dried fish product such as dried anchovy with 27 species belonging to 20 different genera with the dominance of *Staphylococcus aureus*, *A. lwoffii* and *S. warneri*. Ambient storage ($23 \pm 2^\circ\text{C}$, 68% RH) for 12 weeks did not affect the bacterial load, levels of biogenic amines or PV. Traditional dried anchovy was found to be safe microbiologically and to retain good quality for 12 weeks at ambient temperature. The viabi-

ility and absence of pathogens and good storage stability of traditional anchovies at ambient temperature can make this product a reliable source of animal proteins especially in poor developing countries lacking access to electricity and sea.

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