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ORIGINAL ARTICLE

Could halophilic archaea improve the traditional salted anchovies (*Engraulis encrasicholus* L.) safety and quality?

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Abstract

Aims: The positive influence of two selected extremely halophilic archaea strains in the production of salted anchovies (*Engraulis encrasicolus*, L., 1758) was highlighted.
Methods and Results: Anchovies produced with salt artificially contaminated with halophiles exhibited lower loads of staphylococci, *Enterobacteriaceae* and lactic acid bacteria, and a reduced content of histamine as well as an improved organoleptic acceptance.
Conclusions: The findings of this survey are expected to enhance the safety of salted anchovies, with regard to the histamine formation during ripening, and to improve the sensory attributes of this product.
Significance and Impact of the Study: This study represents the first report on the positive influence of halophilic archaea in traditional salted anchovies production, thus suggesting new perspectives about a conscious employment of properly selected haloarchaea strains in this traditional manufacture.

Introduction

Extremely halophilic archaea occur ubiquitously in nature where the salt concentration is high, that is, in salt lakes, soda lakes and salterns (Oren 2002). Although initial interest turned to the involvement of these micro-organisms in the spoilage of salted meat and fish products (Petter 1931; Eddy 1958), systematic studies on the occurrence of such micro-organisms in food ecosystems have rarely been performed.

During the production of salted anchovy (*Engraulis encrasicolus*, L., 1758), a traditional process is used by Mediterranean fishermen to obtain a tender product with a specific, pleasant aroma and taste, a variety of halotolerant and halophilic micro-organisms thrives during and at the end of the process (Villar *et al.* 1985; Caseario and Caramaschi 1993; Hernandez-Herrero *et al.* 2002; Karaçam *et al.* 2002; Moschetti *et al.* 2006), but their role in salting and curing this product has not yet been investigated.

The ripening of salted anchovy is a biochemical process that causes chemical and physicochemical changes in fish tissues and it is widely recognized that it takes places via enzymatic pathways. On the contrary, the relative importance attributed to tissue enzymes *vs* microbial enzymes is still controversial (Hernandez-Herrero *et al.* 2002).

Within anchovy microflora, the extremely halophilic archaea belonging to the *Halobacteriaceae* family are often isolated but their role in salting anchovies and curing has not yet been investigated. Actually, Gram and Huss (1996) reported that these bacteria cause a type of spoilage known as 'pink' condition. Halophilic pink bacteria are, in point of fact, strongly proteolytic and can produce off-odours and -flavours in this product.

In the light of the above, the aim of this study was to elucidate the influence of selected halobacterial strains in the production of salted anchovies according to a traditional procedure. Halobacteria used for this research were isolated during a previous research (Moschetti *et al.* 2006).

Materials and Methods

Salted anchovies experimental manufacturing

Fresh anchovies used for this study were purchased in the local fish market, kept in ice (0·1°C) and transported to the laboratory within 30 min. For the experiments, anchovies (about 3 kg) were beheaded, manually gutted and transferred in tray with granulated salt for 2 h. Then, anchovies were disposed in each of nine different glass jars (diameter 24 cm, height 22 cm, approximately 4 l capacity), according to the traditional procedure named 'head–tail'. The jars were packed with alternate layers of fish and salt (two parts of fish, one part of salt) artificially inoculated with halophilic archaea strains (see below).

For the first 30 days, a glass layer with a 2 kg weight was placed in each jar in order to keep the fish under constant pressure. Successively, a 1 kg weight was used up to the end of the maturation process. Jars were kept for 150 days at 20°C. Each type of experiment was carried out in triplicate, namely three fermentations for each thesis took place on the same days in the same conditions. Samples for both chemical and microbiological analyses were aseptically taken along anchovies ripening.

Preparation of artificial inoculated salt

The procedure followed to prepare the inoculated salt to be employed for the experimental anchovies' manufacturing can be summarized as follows. Commercial saline salt was divided in three lots to prepare saturated saline solutions (1/3 w/v). Two brines were inoculated with two different halophiles cultures previously grown in Halobacterium medium (DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany) at 44°C for 15 days to obtain at least 10^9 CFU ml^{-1}. In detail, strain *Halobacterium salinarium* CER6a, characterized by proteolytic activity on anchovies sarcoplasmatic extract and decarboxylase activity on tyrosine and lysine medium, and strain *Haloarcula marismortui* 1R, with no proteolytic activity on anchovies proteic extracts, were isolated from the state salt pan of Cervia (Ravenna, Italy) and from Sicilian salted anchovies, respectively (Moschetti *et al.* 2006). A third saline solution was prepared without halobacterial strains to obtain a negative control. Saline solutions were treated in heater at 44°C for 10 days under constant lighting with the aim of emulating the natural conditions of solar salterns. A PCR amplification of the archaeobacterial 16S rDNA gene was performed to ascertain the absence of halophilic archaea in uninoculated salt to be used as negative control (Moschetti *et al.* 2006).

Microbiological counts

Counts were performed by spreading plate method on brine developed in the jars during anchovy's maturation. Total aerobic mesophilic micro-organisms, mesophilic lactic acid bacteria (LAB), *Enterobacteriaceae* and *Staphylococcaceae* were counted on Plate Count Agar (30°C for 48 h), MRS agar (overlay agar, 30°C for 48 h), Violet Red Bile Glucose Agar (overlay agar, 30°C for 24 h) and Mannitol Salt Agar (37°C for 48 h), respectively. The media employed were all provided by Oxoid (Basingstoke, UK). Halobacterium medium was used for halophilic populations monitoring (44°C for 15 days under constant conditions of lighting). Samples were taken for analysis at 0, 4, 11, 21, 36, 104 and 136 days of ripening.

The results of microbiological counts were first statistically analyzed by ANOVA. The Student's paired *t*-test was then applied to evaluate statistical significance of the two strains (CER6a and 1R) in comparison to the control. Significant difference was attributed to $P \leq 0{\cdot}05$. Analyses were performed with programs located at http://www.physics.csbsju.edu/stats/t-test.html.

Histamine determination

Determination of histamine was carried out by acid extraction and derivatization using the methods of Moret and Conte (1996) and Galgano *et al.* (2001), respectively.

Sarcoplasmatic protein analysis

Changes in the muscle sarcoplasmatic proteins during the ripening of salted anchovies were examined by 12% SDS–PAGE (Laemmli 1970) on fresh anchovies and after 0, 2, 3, 4, 8, 21, 65 99 days of ripening. By the day 21, sarcoplasmatic extractions were even carried out on the produced brine.

The extraction of sarcoplasmic proteins was performed as described by Molina and Toldrà (1992), with the modifications proposed by Fadda *et al.* (1999).

Sensory analysis

After 3 and 6 months, salted anchovies were washed under running water to remove the excess of salt and subjected to a blind sensory evaluation by a 10-member panel selected and trained under ISO standards (ISO 1993). Seventeen parameters related to flavour and texture (Aspect: compact, dry; Odour: salt anchovies odour, sea odour; Rheological: gummy, juicy; Taste: sweet, acid, bitter, salt, anchovy paste taste, ham taste; Flavour: fresh fish, raw blood, putrid, rancid, other) were assessed using a 10-point quality scale with intensity descriptors at the

end points (1, low; 10, high). Samples were three-digit coded and the order of serving was determined by random permutation. Two panel replications were carried out on each sample. An additional hedonic test was performed to evaluate the overall acceptability.

The sensory data for each attribute were submitted to one-way ANOVA, considering as an independent variable the days of maintenance and as a dependent variable the sensory attribute. Significance was tested with the *F* test. The mean values were submitted to the multiple comparison test using the least significant difference (LSD) procedure that allows the attributes which differentiate the samples to be determined.

Results

Enumeration of micro-organisms during anchovies ripening

Microbial counts on Halobacterium medium revealed, in the two contaminated salt, a population of about 10^4 CFU g^{-1} for both archeal strains employed, while in salt used as negative control halophiles were at undetectable level (<10). Moreover, the PCR amplification of the archaeobacterial 16S rDNA gene confirmed the absence of DNA from archaeal origin in uninoculated salt to be used as negative control (Fig. 1). The results of the viable counts of the targeted microbial groups are reported in Table 1. At time zero counts ranged between 10^2 and 10^3 CFU ml^{-1} for all the media considered. After 4 days of maturation in the control manufacture with uninoculated salt, the loads on Plate Count Agar increased above 10^5 CFU ml^{-1} and, except for a slight drop at 36 days, continued to grow up to the end of ripening (10^6 CFU ml^{-1}) (Table 1). By contrast, in anchovies obtained by using salt inoculated with halobacteria strains, mesophilic aerobic bacteria remained around the initial number (10^3 CFU ml^{-1}) up to the end of ripening. LAB on MRS agar exhibited a similar trend: loads in samples produced without halophilic archaea were more then one decade higher in the product ready to be consumed (Table 1). *Enterobacteriaceae* on VRBGA decreased along the ripening period in all cases, even if declining started early in samples treated with halobacteria. Staphylococci proved to be strongly inhibited by the presence of archaea representatives in the salt: at day 104, counts on control samples reached values of almost 10^6 CFU ml^{-1}, whereas loads in samples produced with halobacteria contribution showed a noticeable fall (Table 1). In general terms, in samples treated with strain Cer6A were recorded the lowest counts. Halophiles counts did not significantly change during ripening and were comprised between 2 and 3 log CFU ml^{-1} (Table 1).

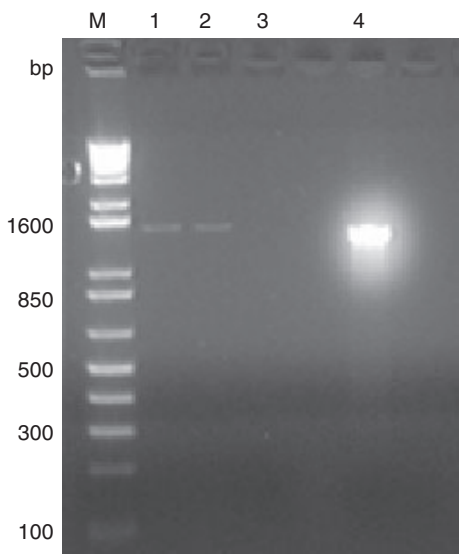

Figure 1 Ethidium bromide-stained 1% agarose gel of PCR-amplified archaeobacterial 16S rDNA. Line 1, salt enriched with *Haloarcula marismortui* strain 1R; line 2, salt enriched with *Halobacterium salinarum* strain CER6a; line 3, salt used as negative control; line 4, DNA extracted by *Halobacterium salinarum* strain CER6a; line M 1 Kb DNA Ladder (Invitrogen SRL) used as molecular weight marker.

Histamine levels during anchovies ripening

Figure 2 reports the histamine levels during anchovies maturation. In anchovies ready to be consumed, histamine levels are less then 100 ppm in all cases but significantly different trends could be detected during the first 21 days of anchovies maturation. In control samples produced with uninoculated salt, histamine levels reached values approximately 580 ppm after 11 days of ripening. At the same time, loads on PCA and MRS media reached the maximum of population level (approximately 5 log CFU ml^{-1}).

Sarcoplasmatic proteins evolution during anchovies ripening

Proteolysis occurring during ripening of salted anchovies was followed by SDS–PAGE of sarcoplasmatic proteins. Because the salting process affected the resolution of protein bands (Greaser *et al.* 1983), proteolysis was followed by monitoring the sole major bands of each pattern. Proteins of molecular weight higher than 37 kDa disappeared during the salting process and after 8 days of ripening sarcoplasmatic proteins were totally denatured (data not shown). By contrast, profiles obtained by liquid generate along ripening showed an increasing number of bands (data not shown).

Table 1 Changes in microbial counts (log CFU ml^{-1} or g^{-1} and SD) during ripening of salted anchovies

		Days of ripening						
Media and incubation conditions		0	4	11	21	36	104	136
PCA (30°C, 48 h)	Strain CER6a	2·32 ± 1·70^{a}	2·10 ± 1·55^{b}	1·99 ± 1·46^{b}	2·50 ± 1·46^{b}	1·37 ± 0·76^{b}	2·68 ± 2·18^{b}	2·69 ± 1·91^{b}
	Strain 1R	3·16 ± 0·81^{a}	4·53 ± 3·41^{a}	2·73 ± 1·76^{b}	2·52 ± 1·74^{b}	2·19 ± 1·49^{b}	2·90 ± 2·19^{b}	2·89 ± 1·98^{b}
	Control	3·96 ± 1·11^{a}	5·18 ± 3·79^{a}	5·70 ± 4·85^{a}	5·56 ± 4·98^{a}	4·72 ± 3·39^{a}	6·24 ± 5·36^{a}	6·27 ± 5·09^{a}
MRS (overlay agar, 30°C, 48 h)	Strain CER6a	2·59 ± 1·75^{a}	1·43 ± 1·39^{a}	1·22 ± 0·76^{b}	1·80 ± 1·71^{b}	1·64 ± 1·46^{b}	2·05 ± 1·77^{b}	2·07 ± 1·55^{b}
	Strain 1R	2·52 ± 1·61^{a}	1·56 ± 1·49^{a}	2·42 ± 2·00^{b}	1·56 ± 1·49^{b}	1·67 ± 1·06^{b}	2·08 ± 1·24^{b}	2·04 ± 1·00^{b}
	Control	2·57 ± 1·48^{a}	1·82 ± 1·49^{a}	5·53 ± 4·74^{a}	4·45 ± 3·78^{a}	4·13 ± 3·62^{a}	3·49 ± 2·64^{a}	3·47 ± 2·18^{a}
VRBGA (overlay agar, 30°C, 24 h)	Strain CER6a	2·71 ± 2·41^{a}	2·66 ± 2·08^{a}	2·39 ± 1·32^{a}	2·30 ± 1·91^{a}	1·14 ± 1·17^{a}	0·87 ± 1·04^{a}	0·78 ± 0·42^{a}
	Strain 1R	2·85 ± 2·56^{a}	1·85 ± 1·24^{a}	2·10 ± 1·88^{a}	2·22 ± 1·75^{a}	1·37 ± 0·76^{a}	0·85 ± 0·72^{a}	0·56 ± 0·32^{a}
	Control	2·73 ± 2·40^{a}	2·59 ± 2·56^{a}	2·98 ± 2·40^{a}	2·81 ± 2·41^{a}	2·28 ± 2·03^{a}	1·14 ± 1·17^{a}	0·94 ± 0·18^{a}
MSA (37°C, 48 h)	Strain CER6a	1·18 ± 0·87^{a}	2·52 ± 1·64^{a}	2·17 ± 1·62^{a}	2·01 ± 1·40^{b}	1·95 ± 1·95^{b}	0·52 ± 0·76^{b}	nd
	Strain 1R	2·47 ± 1·93^{a}	3·83 ± 3·31^{a}	3·30 ± 2·64^{a}	3·71 ± 2·89^{a}	3·68 ± 2·18^{a}	1·95 ± 1·42^{b}	1·69 ± 0·71^{b}
	Control	2·21 ± 1·02^{a}	3·78 ± 3·63^{a}	3·88 ± 2·54^{a}	3·83 ± 2·64^{a}	4·26 ± 3·67^{a}	5·75 ± 5·14^{a}	5·77 ± 4·96^{a}
Halobacterium medium (44°C, 15 days)	Strain CER6a	3·80 ± 1·00^{a}	3·44 ± 2·42^{b}	2·88 ± 1·67^{a}	2·91 ± 2·12^{a}	2·54 ± 1·85^{a}	2·79 ± 2·02^{a}	2·69 ± 1·97^{a}
	Strain 1R	3·24 ± 1·30^{a}	2·56 ± 1·32^{a}	2·66 ± 1·81^{a}	2·45 ± 1·85^{a}	2·48 ± 1·78^{a}	2·61 ± 1·69^{a}	2·54 ± 1·32^{a}
	Control	nd	nd	nd	nd	nd	nd	nd

nd, not detected. Means along a column with a different superscript letter (a, b) were significantly different ($P < 0·05$).

Figure 2 Histamine values (ppm) observed during anchovies maturation with salt with and without halobacteria strains contribute.

Sensorial analysis

Finally, anchovies were submitted to sensorial analysis at 3 and 6 months of ripening. At the first sampling, sensory descriptors did not appear remarkably affected by the presence of halophiles in the salt, except the attribute 'Putrid' and 'Raw blood' that was superior in the control fermentation (Fig. 3). Further significant differences emerged with reference to factors perceived by tongue: anchovies manufactured with halophilic starter showed a higher taste of anchovy paste. On the contrary, no atypical flavour was recorded in the control production, whereas the descriptor 'Other' resulted higher in anchovies produced with both archaeal strains (Fig. 3). After 6 months, samples revealed noteworthy differences. Anchovies produced with inoculated salt were more 'Juicy', less 'Bitter', 'Acid' and 'Dry', and with a strongest aroma of typical anchovies and of sea. Anchovies of the control lot exhibited a higher taste of 'Putrid', 'Rancid', 'Raw blood' and an elevated value for the descriptor 'Other' (Fig. 3). With regard to the overall acceptability, anchovies produced with inoculated salt resulted largely more pleasant, above all, as expected, in the panel test performed after 6 months of ripening (Fig. 4).

Discussion

Histamine poisoning (scombroid fish poisoning or scombro toxicosis) is a foodborne chemical intoxication resulting from the ingestion of foods that contain high levels of histamine (Sumner and Taylor 1989). During the ripening of salted anchovy, an important proteolysis is observed, with liberation of peptides and free amino acids. When free histidine is found in a sufficient quantity, it can be degraded by the micro-organisms or their enzymes. Consequently, histamine may be formed at this time, and eventually reach toxic levels (Veciana-Nogués *et al.* 1989; Hernandez-Herrero *et al.* 1999).

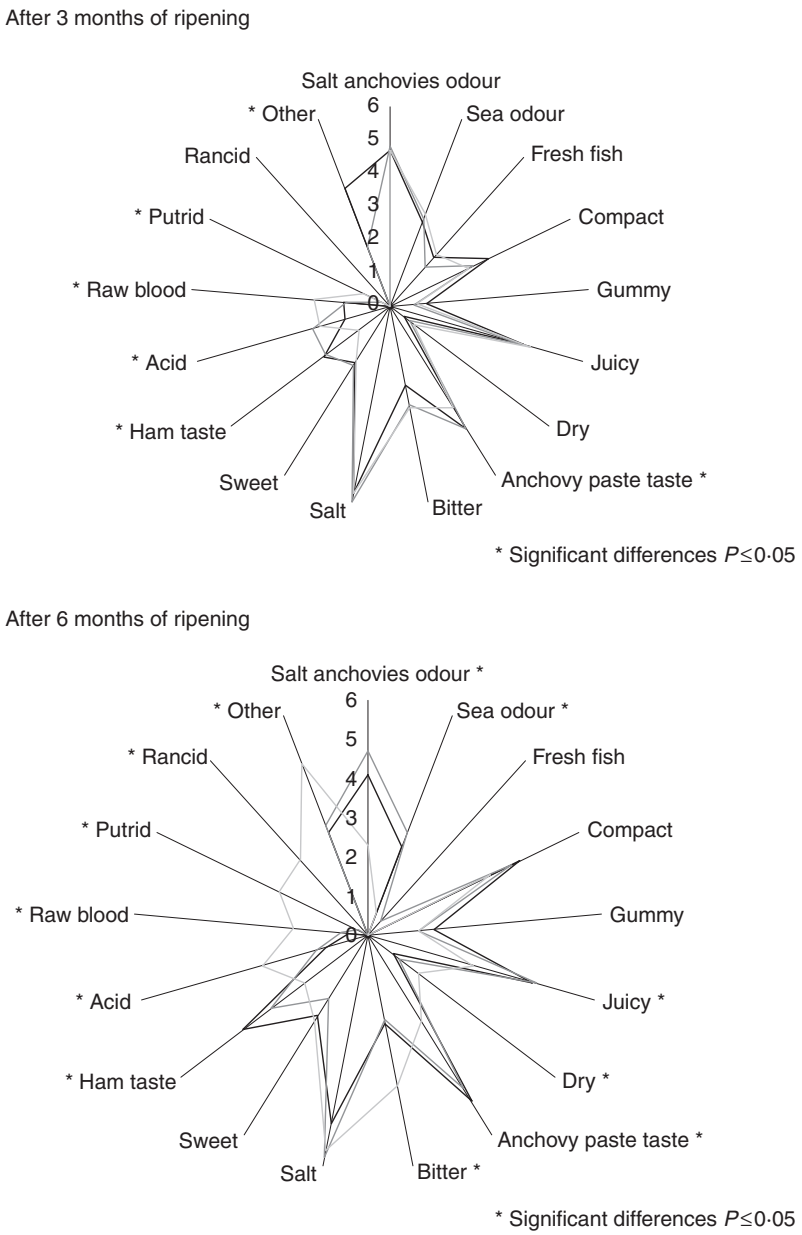

Figure 3 Average grades for the main sensorial characteristics for the three anchovie productions after 3 and 6 months of ripening.

In the present survey, the histamine contents, at the end of salted anchovies monitoring, were, in all trials, significantly lower than the ones reported by other authors. Veciana-Nogués *et al.* (1989), in semi-preserved anchovies observed a maximum of histamine level of 210 ppm after 8 months of storage at ambient temperature and in a succeeding work on the same type of product (Veciana-Nogués *et al.* 1997) even 3000 ppm after 6 months of storage. Likewise, Karaçam *et al.* (2002) found that at ambient temperature, histamine values in brined anchovies reached EU limits at the 19th day of storage and increased on further storage.

The inhibitory effect of starter cultures on histamine formation could be related to competition with biogenic amine producers as well as to a potential capability of biogenic amines degradation; in point of fact, histamine degrading activity in halophilic archeae has been lately reported (Tapingkae *et al.* 2010a,b).

On the contrary, no difference in the hydrolysis rate of muscle sarcoplasmatic proteins emerged by comparing SDS–PAGE profiles of anchovies produced by using the three different types of salt. According to other authors' findings (Hernández-Herrero *et al.* 2000), proteins of molecular weight higher than 37 kDa disappeared during

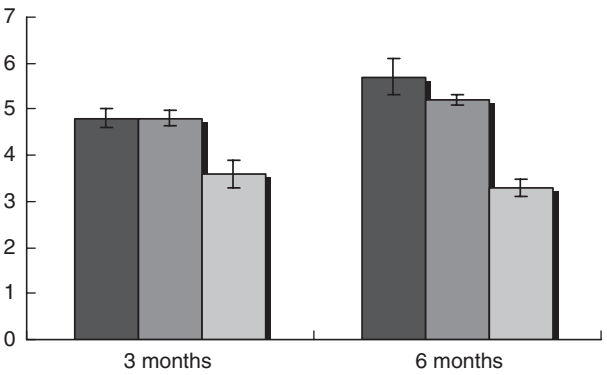

Figure 4 The hedonic score for overall acceptability of the different anchovies productions at 3 and 6 months of ripening.

the salting process and after approximately 1 week of ripening sarcoplasmatic proteins were totally denatured.

In conclusion, several works report the occurrence of new archeal species in fish products (Namwong *et al.* 2007; Yachai *et al.* 2008; Namwong *et al.* 2010), but just one (Akolkar *et al.* 2009) deals with the potential contribute of halophiles to the improving of fish preservation techniques salt-based under a sensorial and hygienic point of view. The results obtained during this study highlight the potential technological contribute of the halobacterial archaea entities in the salt employed to the traditional salted anchovy manufacturing. In detail, *Haloarcula marismortui* 1R could be considered a suitable candidate for a really innovative protective culture to be proposed in the traditional salted anchovies production.

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References

Akolkar, A.V., Durai, D. and Desai, A.J. (2009) *Halobacterium* sp. SP1(1) as a starter culture for accelerating fish sauce fermentation. *J Appl Microbiol* **109**, 44–53.

Caseario, G. and Caramaschi, N. (1993) La microbiologia delle semiconserve ittiche: acciughe sott'olio. *Industria Conserve* **68**, 142–147.

Eddy, B.P. (1958) *The Microbiology of Fish and Meat Curing Brines*, Proc. 2nd. Int Symp *Food Microbiol*. H.M. Stat, Office, London.

Fadda, S., Sanz, Y., Vignolo, G., Aristoy, M.C., Oliver, G. and Toldrà, F. (1999) Hydrolysis of pork muscle sarcoplasmatic proteins by *Lactobacillus curvatus* and *sakei*. *Appl Environ Microbiol* **65**, 578–584.

Galgano, F., Suzzi, G., Favati, F., Caruso, M., Martuscelli, M., Gardini, F. and Salzano, G. (2001) Biogenic amines during ripening in 'Semicotto Caprino' cheese: role of enterococci. *Int J Food Sci Technol* **36**, 153–160.

Gram, L. and Huss, H.H. (1996) Microbiological spoilage of fish and fish products. *Int J Food Microbiol* **33**, 121–137.

Greaser, M.L., Yates, L.D., Krzywicki, K. and Roelke, D.L. (1983) Electrophoretic methods for the separation and identification of muscle proteins. *Reciprocal Meat Conference Proceedings* **36**, 87–91.

Hernandez-Herrero, M.M., Roig-Sagues, A.X., Rodriguez-Jerez, J.J. and Mora-Ventura, M.T. (1999) Halotolerant and halophilic histamine-forming bacteria isolated during the ripening of salted anchovies (*Engraulis encrasicholus*). *J Food Protect* **62**, 509–514.

Hernandez-Herrero, M.M., Roig-Sagues, A.X., Lopez-Sabater, E.I., Rodriguez-Jerez, J.J. and Mora-Ventura, M.T. (2002) Influence of raw fish quality on some physicochemical and microbial characteristics as related to ripening of salted anchovies (*Engraulis encrasicholus L*). *J Food Sci* **67**, 2631–2640.

Hernández-Herrero, M.M., Roig-Sagués, A.X., López-Sabater, E.I., Rodríguez-Jerez, J.J. and Mora-Ventura, M.T. (2000) SDS-PAGE of salted anchovies (*Engraulis encrasicholus* L) during the ripening process. *Eur Food Res Technol* **212**, 26–30.

ISO. (1993). *ISO 8586-1, Sensory Analysis. General Guidance for Selection, Training and Monitoring of Assessors*. Switzerland: International Organization for Standardization.

Karaçam, H., Kutlu, S. and Köse, S. (2002) Effect of salt concentrations and temperature on the quality and shelf-life of brined anchovies. *Int J Food Sci Technol* **37**, 19–28.

Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **277**, 680–685.

Molina, I. and Toldrà, F. (1992) Detection of proteolytic activity in microorganisms isolated from dry cured ham. *J Food Sci* **57**, 1308–1310.

Moret, S. and Conte, L. (1996) High-performance liquid chromatographic evaluation of biogenic amines in foods. An analysis of different methods of sample preparation in relation to food characteristics. *J Chromatography A* **729**, 363–369.

Moschetti, G., Aponte, M., Blaiotta, G., Casaburi, A., Chiurazzi, M., Ventorino, V. and Villani, F. (2006) Characterization of halophilic Archaea isolated from different hypersaline ecosystems. *Ann Microbiol* **56**, 119–127.

Namwong, S., Tanasupawat, S., Visessanguan, W., Kudo, T. and Itoh, T. (2007) *Halococcus thailandensis* sp. nov., from fish sauce in Thailand. *Int J Syst Evol Microbiol* **57**, 2199–2203.

Namwong, S., Tanasupawat, S., Kudo, T. and Itoh, T. (2010) *Haloarcula salaria* sp. nov. and *Haloarcula tradensis* sp. nov. from salt in Thai fish sauce. *Int J Syst Evol Microbiol*. In press. Doi: 10.1099/ijs.0.021790-0.

Oren, A. (2002) Molecular ecology of extremely halophilic Archaea and Bacteria. *FEMS Microbiol Ecol* **39**, 1–7.

Petter, H.F.M. (1931) On bacteria of salted fish. *Proc. Konink, Akad, Wet. Amsterdam. Ser. B.* **34**, 1417–1423.

Sumner, S.S. and Taylor, S.L. (1989) Detection method for histamine-producing, dairy related bacteria using diamine oxidase and leucocrystal violet. *J Food Protect* **52**, 105–108.

Tapingkae, W., Parkin, K.L., Tanasupawat, S., Kruenate, J., Benjakul, S. and Visessanguan, W. (2010a) Whole cell immobilisation of *Natrinema gari* BCC 24369 for histamine degradation. *Food Chem* **120**, 842–849.

Tapingkae, W., Tanasupawat, S., Parkin, K.L., Benjakul, S. and Visessanguan, W. (2010b) Degradation of histamine by extremely halophilic archaea isolated from high salt-fermented fishery products. *Enz Microb Tech* **46**, 92–99.

Veciana-Nogués, M.T., Vidal-Carou, M.C. and Mariné-Font, A. (1989) Histamine and tyramine in preserved and semi-preserved fish products. *J Food Sci* **54**, 1653–1655.

Veciana-Nogués, M.T., Mariné-Font, A. and Vidal-Carou, M.C. (1997) Changes in biogenic amines during the storage of Mediterranean anchovies immersed in oil. *J Agric Food Chem* **45**, 1385–1389.

Villar, M., de Ruiz Holgado, A.P., Sanchez, J.J., Trucco, R.E. and Oliver, G. (1985) Isolation and characterization of *Pediococcus halophilus* from salted anchovies (*Engraulis anchoita*). *Appl Environ Microbiol* **49**, 664–666.

Yachai, M., Tanasupawat, S., Itoh, T., Benjakul, S., Visessanguan, W. and Valyasevi, R. (2008) *Halobacterium piscisalsi* sp. nov., from fermented fish (pla-ra) in Thailand. *Int J Syst Evol Microbiol* **58**, 2136–2140.