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PRESERVATION OF BLUE-JACK MACKEREL (*TRACHURUS PICTURATUS* BOWDICH) SILAGE BY CHEMICAL AND FERMENTATIVE ACIDIFICATION

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ABSTRACT

We compared acidified and lactic acid fermented silage approaches for the preservation of blue-jack mackerel. Silages acidified with formic and propionic acids had stable pH (3.8) and low (19 mg/g N) levels of volatile nitrogen compounds (total volatile basic nitrogen, TVBN), but relatively high (82 g/100 g) final non-protein-nitrogen (NPN) values.

*The silage was fermented with *Lactobacillus plantarum* LU853, a homo-fermentative lactic acid bacterium with a high growth (0.51/h) and acidification rate at 37°C (optimum temperature), able to grow in the presence of 40 g/L NaCl and to ferment sucrose and lactose. The silages at 37°C reached safe pH < 4.5 values within 48–72 h, either (F2a) or not (F0), in combination with 20 g/kg salt addition; F2a acidified more rapidly, which may be an advantage for its microbiological stability. Proteolysis resulting in 53–59 g NPN/100 g N was lower in fermented than in acidified silages; however, in fermented silages, the levels of TVBN were much higher (50–80 mg TVBN/g N) than generally considered acceptable.*

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PRACTICAL APPLICATIONS

Preservation of underutilized fish/fish waste by acidification would provide a sustainable means of preserving a valuable, increasingly scarce resource. Such fish silages may be used as a component of animal feeds, as a fertilizer or as a source for the extraction of bioactive compounds, such as enzymes that are suitable for gentle processing at low temperatures or bioactive peptides (e.g., gastrins, cholecystokinins and peptides with opioid-like activity). These compounds may be used for enhancing growth and disease resistance of animals, and possibly in the pharmacological industries. Fish silages may also be used as culture media components for the cultivation of microorganisms.

INTRODUCTION

In spite of its increasing scarcity, fish catch is not always fully utilized because of premature spoilage. In addition, fish processing generates by-products that require preservation. Transformation and preservation through silage has been considered (Lindgren and Pleje 1983) as the most suitable procedure for recycling wastes and by-products from the fish processing industry. Fish silages are mainly produced for animal feeds (Raa and Gildberg 1982), for human consumption (Mackie *et al.* 1971) and as plant fertilizers (Synnes and Opstad 1995). Silages are regarded as stable when their pH is below 4.5 (Lassen 1995b). In principle, acidification can be achieved by adding acids (here referred to as "acidified silage") or by fermentation with lactic acid bacteria (LAB) ("fermented silage"). Considerable deterioration can occur while the pH is still >4.5. Proteolysis can be a problem, particularly when it leads to excessive formation of volatile nitrogen compounds, offensive off-odors, undesirable formation of gas and decreased nutritional value (Raa and Gildberg 1982). The critical period during which the pH of the silages is above 4.5 must be kept as short as possible. According to Lindgren and Pleje (1983), the pH decrease to values below 4.5 must be completed within 48–72 h to ensure silage stability and safety.

Fish preservation by addition of acids has been described (Raa and Gildberg 1982); when using organic acids, a target pH of 3.5–4.0 for adequate preservation could be obtained using a mix of acetic (20 g/kg), formic (15 g/kg), propionic (4 g/kg), benzoic (4 g/kg) and sorbic (1 g/kg) acids. Although acid addition was reported to result in stable silages, independent of temperature, they could still permit mold growth at their surface (Raa and Gildberg 1982).

LAB, in very small numbers, are part of the natural microflora of fish (Raa *et al.* 1983). However, inoculating the fish pastes intended for fish silage production with a suitable culture of LAB will greatly enhance the acidification. A starter LAB culture must be able to ferment the added carbohydrate(s) by the homofermentative pathway in order to produce as much lactic acid as possible while avoiding gas production (Mackie *et al.* 1971); it must have a high growth rate at the intended temperature range and compete with the fish native microflora (Adams *et al.* 1987). At the Azores, the ambient temperature range is 17–20°C; from an energy-saving point of view, it would be of interest to preserve the fish at this temperature. Optimum temperatures for lactic acid fermentation of fish silage are 35–40°C, and we used 37°C as an optimum temperature in our comparative tests.

To avoid spoilage of the silages, the duration of the critical period until the pH decreases below 4.5 should be minimized by incubation at optimum temperature or by incorporation of additives that inhibit undesirable microorganisms. Gram-negative bacteria of the genera *Pseudomonas* and the *Acinetobacter-Moraxella* group predominate among the microflora of deteriorating fish (Jackson *et al.* 1997). Because these bacteria are sensitive to NaCl, salt could be used to protect the silages during early stages of fermentation and to ensure their subsequent preservation. The buffering capacity of fish pastes is especially high within pH 5–6 (Adams *et al.* 1987). Therefore, considerable time (with chances of deterioration) may elapse before a safe pH < 4.5 has been achieved (Owens and Mendoza 1985). Decreasing the initial pH of the silages by adding organic acids could complement LAB action during this initial period. Combinations of NaCl and low initial pH could lead to stronger inhibition of undesirable microorganisms (Schabolt *et al.* 2001; Koutsoumanis *et al.* 2003). Under conditions of oxygen access, mold growth can occur on the silage surfaces, leading to deterioration and even to mycotoxin formation. Propionic acid has been used to control mold growth on the exposed surfaces of fish silages (Johnsen and Skrede 1981; Strom and Eggum 1981; Lindgren and Pleje 1983).

Blue-jack mackerel is abundant and fetches low prices in the Azores, resulting in occasional dumping of unused catch of this species in the municipal landfills. Whereas our focus was not on comparisons with other fish species, we aimed at comparing preservation options for blue-jack mackerel silage, including the above-mentioned approaches to shorten the critical acidification period and to protect silages against undesirable microorganisms, with additives. In addition to comparing acidified and fermented silages, we also assessed the effects of pH adjustments and of the addition of salt and propionic acid on the microbiological stability, proteolysis and physicochemical properties of fish silage.

MATERIALS AND METHODS

Strains

Several *Lactobacillus* spp., isolated from naturally fermented foods and feeds, were tested for their potential applicability as silage inoculum. These included *Lactobacillus plantarum* LU849, LU850, LU855 and LU856 (isolated from grass silages [Bonestroo *et al.* 1992]); *Lb. plantarum* LU853, LU854 and *Lb. casei* LU852 (isolated from tempe soak water ([Nout *et al.* 1987])); *Lb. delbrückii* CUC-I; and *Lactobacillus* sp. LPS-20 and LPCD-8 (isolated from olives). LU strains were provided by the Laboratory of Food Microbiology of Wageningen University, the Netherlands; the other strains were obtained from the Technical University of Lisbon, Portugal. The strains were maintained in de Man, Rogosa and Sharpe (MRS) agar (MRS broth with 15 g/L added Bacto Agar; Pronadisa, 1056, Hispanlab, Madrid, Spain) as stab cultures, at 4C. Overnight (12–16 h) cultures were obtained prior to use as silage starter, by subculturing three times in MRS broth (Merck 1066, Darmstadt, Germany).

Properties of the Isolates

Growth at 17 and 37C, and growth in the presence of 40 g/L NaCl were monitored in MRS broth by measuring the optical density at $\lambda = 620$ nm with a blank of sterile MRS broth. Measurements were taken at 1-h intervals during the first 24 h; the isolates incubated at 17C were measured during three consecutive days. The pH was measured directly in samples of the broth cultures (Nout *et al.* 1989). Ability to ferment sucrose and lactose was judged from the acidification in modified MRS in which glucose was replaced by these sugars, and chlorophenol red was added (0.4 g/L) as pH indicator. All tests were performed in triplicate.

Fish Silage

Fresh blue-jack mackerel (average length, 38 cm) (*Trachurus picturatus* Bowdich) was purchased at the local market in Angra do Heroísmo, Azores, Portugal, and was immediately used for silage preparation. Whole – not eviscerated – fish were cut into pieces and ground in a meat grinder through a 4-mm diameter die.

The variables under study were as follows: sucrose addition (10 and 12%); NaCl addition (0, 2 and 4%); addition of 3% formic acid + 0.75% propionic acid at time 0; and addition of 0.75% formic acid (added after completion of fermentation). The acidification treatments were carried out only on the fish silages with 12% added sucrose +2% added NaCl.

TABLE 1.
COMPOSITION OF EXPERIMENTAL SILAGES OF BLUE-JACK MACKEREL
(*TRACHURUS PICTURATUS*)

Treatment	GRF (kg)	Water (kg)	Sucrose (kg)	NaCl (kg)	Formic acid (kg)	Propionic acid (kg)	HCl	Starter* (kg)	Initial pH
A	8.625	1.0	—	—	0.3	0.075	—	—	3.8
F0	7.7	1.0	1.2	—	—	—	—	0.1	6.7
F2a	7.5	1.0	1.2	0.2	—	—	—	0.1	6.6
F2b	7.5	1.0	1.2	0.2	—	—	tr†	0.1	5.1
F2c	7.425	1.0	1.2	0.2	—	0.075‡	—	0.1	6.5
F4	7.3	1.0	1.2	0.4	—	—	—	0.1	6.6

* *Lactobacillus plantarum* LU 853 culture grown 12–16 h at 30C, achieving initial inoculation level of 10^6 cfu/g silage.

† Trace level to adjust pH to 5.1; ‡ added after completion of fermentation.

A, acidified silage; F0, fermented silage, with numbers indicating salt levels, with a: standard, b: pH adjusted to 5.1 and c: added propionic acid; GRF, ground raw fish.

Table 1 shows the composition of the different types of silages. Tapwater was added in order to obtain a pasty consistency. Added ingredients (sucrose, NaCl, formic acid and propionic acid as required) were dissolved or mixed in the water prior to preparation of the fish paste. The level of sucrose (120 g/kg) was quite high but effective, and was initially based on studies using molasses (Dong *et al.* 1993). Cane molasses were added (Dong *et al.* 1993) to fish for ensiling at levels of 40–300 g/kg. These authors did not state the sugar concentration in the molasses they used. The average sucrose content of molasses is about 470 g/kg, so sucrose was present at 20–140 g/kg. We found (Dapkevicius *et al.* 1998), however, that 200 g/kg molasses addition (corresponding to approximately 94 g/kg sucrose) did not lead to a pH below 4.5 within the required 48 h. For this reason, a higher sucrose concentration was applied in the present work. For acidified silage (A), we simplified the mix of organic acids (Raa and Gildberg 1982), as mentioned in the Introduction, to include only 30 g/kg formic acid and 7.3 g/kg propionic acid, aiming at a silage pH of 3.8.

For each treatment, a batch of 10 kg was prepared by manual paddle-mixing during 15 min, and was distributed into jars (300 g/500-mL glass screw-capped jar), so that four jars per treatment per day were available. Thus, 28 jars (four jars for each sampling day) were prepared per treatment. Any remaining mixture was discarded.

To each jar of silage for fermentation, 10 g/kg of 12- to 16-h-old MRS broth cultures of *Lb. plantarum* LU 853 was added, giving initial counts of 10^6 – 10^7 cfu/g. After inoculation, the pastes were thoroughly mixed and the jars

were sealed to exclude air. Incubation was carried out in an incubator (37C) and at room temperature (17 ± 1 C).

Microbiological Analysis

At each sampling time (days 0, 1, 2, 3, 7, 14 and 21), four jars of each treatment were opened and sampled for pH measurement and microbiological analysis. After thorough mixing by spatula, 20 g of ensiled paste was aseptically collected. Appropriate decimal dilutions were prepared in sterile peptone physiological saline (1 g/L bacteriological peptone and 9 g/L NaCl in demineralized water). The LAB were enumerated on MRS agar (CM 361, Oxoid, Hampshire, England) pour plates containing 1 g/L natamycin (Delvacid, DSM, Delft, the Netherlands) after incubation for 5 days at 30C in an anaerobic jar (Anaerocult A, Merck KGaA). Coliform bacteria were plated on Violet Red Bile Agar (0012-01-5, Difco, Detroit, MI) pour plates with an overlay of the same medium, incubated at 37C for 24 h. Yeasts and molds were enumerated on Potato Dextrose Agar spread plates (CM 139, Oxoid, Basingstoke, U.K.) acidified to pH 3.5 with a 100 g/L tartaric acid solution, after incubation at 25C for 5 days. Heat-resistant aerobic bacterial spores were enumerated on pour plates of Plate Count agar (0479-01-1, Difco) after inactivation of the vegetative cells at 80C during 5 min. All analyses were performed in quadruplicate.

Chemical Analysis

For the determination of non-protein nitrogen (NPN), 25 g of silage was homogenized for 5 min with 150 mL of 7% trichloroacetic acid and was filtered through Whatman No. 1 paper (Batista *et al.* 1987); the nitrogen content of the filtrate was determined by a micro-Kjeldahl procedure (AOAC 1990). Total volatile basic nitrogen (TVBN) was determined by the Conway microdiffusion method (Anon 1988). All analyses were performed in quadruplicate.

Water-binding Capacity/Moisture-releasing Properties of Silages (Silage Moisture Release, SMR)

Fifteen-gram samples of silage were thoroughly mixed and centrifuged at $25,000 \times g$ for 30 min at 12C in a refrigerated centrifuge (DAMON, B-20A, Damon/IEC Division, Needham, MA). The supernatant was discarded and the sediment was weighed. The SMR (g/100 g) was calculated as $100 - (\text{weight of sediment/weight of the initial sample}) \times 100$.

Statistical Analysis

Rates of growth and acidification were obtained by fitting equations by linear regression (least squares method) to the growth and pH data obtained

during screening tests of the LAB cultures. Separation of the means was performed by the Duncan test (Snedecor and Cochran 1980).

RESULTS AND DISCUSSION

Screening of LAB Cultures

All strains had been selected on the basis of their homofermentative metabolism. Table 2 lists the strains according to increasing growth rate. The rates of growth and acidification were not necessarily correlated. For practical applications, the abilities to ferment sucrose as well as lactose, and the ability to grow in the presence of salt and at a broad range of temperatures were considered to be important selection criteria. On the basis of its versatility, *Lb. plantarum* LU853 was selected for experimental fish silages.

Effect of Silage Treatments on Acidification and Microbiological Composition

Tables 3 and 4 show the results of experiments with ground fish preserved by acid addition (A) and ground fish that was fermented (F) under experimental conditions. The obvious characteristic of the acidified silage (A) was its low pH (3.6–3.8) throughout the experiment, which was adjusted to the recommendations made elsewhere (Raa and Gildberg 1982), and which was lower than the pH range (4.0–4.5) obtained in successfully fermented silages (Lassen 1995a; Ahmed and Mahendrakar 1996). Table 3 shows that this treatment is microbiologically stable.

Of the fermented ground fish silages, the control formulation F0 was tested at both 17 and 37°C. The data in Tables 3 and 4 indicate that at 37°C, a significant growth of LAB with concomitant disappearance of coliforms and yeasts and molds took place, resulting in a lowering of the pH to <4.5 within 2 days. In contrast, at 17°C, the LAB growth was much slower and it took 7 days to lower the pH to 4.5 (data not shown). This slower acidification allowed the proliferation of Gram-negative bacteria such as coliforms, as well as yeasts and molds. We therefore considered the fermentation at 17°C inadequate, and other formulations were tested at 37°C only.

Addition of 20 g/kg salt (F2a) did not significantly affect the LAB growth, but the pH decreased more rapidly than in F0. This combination of salt and rapid acidification reduced the numbers of coliform bacteria, as well as yeasts and molds, effectively. When the silage process was started by adjusting the pH to 5.1 (F2b), the microbial growth was strongly restricted; the LAB remained at inoculum level, whereas levels of coliform bacteria and yeasts and molds were similar as in acidified silage (A). The addition of propionic acid

TABLE 2.
PROPERTIES OF POTENTIAL FISH SILAGE STARTER STRAINS OF LACTIC ACID BACTERIA

Strains	Growth rate (optical density/h)*	Acidification rate (pH/h)*	Sucrose fermentation†	Lactose fermentation†	Growth in 40 g/kg ⁻ NaCl‡	Growth at 17C‡
<i>Lactobacillus</i> sp. LPCD-8	0.30 ± 0.01 ^c	0.19 ± 0.00 ^{ef}	—	—	+	+
<i>Lactobacillus plantarum</i> LU856	0.31 ± 0.04 ^{bc}	0.25 ± 0.01 ^d	+	+	+	(+)
<i>Lactobacillus</i> sp. LPS-20	0.32 ± 0.01 ^{bc}	0.16 ± 0.01 ^f	+	—	+	+
<i>Lb. plantarum</i> LU850	0.42 ± 0.02 ^{abc}	0.33 ± 0.01 ^b	+	+	+	—
<i>Lb. plantarum</i> LU855	0.45 ± 0.06 ^{abc}	0.20 ± 0.02 ^e	+	+	+	(+)
<i>Lactobacillus casei</i> LU852	0.45 ± 0.02 ^{abc}	0.23 ± 0.02 ^d	+	—	—	(+)
<i>Lactobacillus delbrueckii</i> CUC-1	0.51 ± 0.07 ^{ab}	0.23 ± 0.02 ^d	+	—	+	+
<i>Lb. plantarum</i> LU853	0.51 ± 0.03 ^a	0.23 ± 0.01 ^d	+	+	+	+
<i>Lb. plantarum</i> LU849	0.52 ± 0.03 ^a	0.28 ± 0.01 ^a	+	—	+	—
<i>Lb. plantarum</i> LU854	0.76 ± 0.04 ^d	0.28 ± 0.01 ^a	—	—	—	(+)

All tests were carried out in de Man, Rogosa and Sharpe broth at 37C unless mentioned otherwise.

* The numbers represent means ± SD ($n = 3$); within each column, means marked with different letters are significantly ($P < 0.05$) different.

† +, acidification; —, no acidification.

‡ +, growth; (+), weak or delayed growth; —, no growth.

TABLE 3.
MICROBIOLOGICAL CHANGES DURING EXPERIMENTAL SILAGES AT 37°C OF
BLUE-JACK MACKEREL WITH *LACTOBACILLUS PLANTARUM* LU853

	Period (d)	Treatment					
		A	F0	F2a	F2b	F2c	F4
LAB (log cfu/g)	0	<1.85	5.4 ± 0.0 ^a	5.2 ± 0.0 ^b	5.3 ± 0.4 ^{ab}	5.3 ± 0.1 ^{ab}	5.4 ± 0.1 ^a
	1	<1.85	6.6 ± 0.1 ^a	6.7 ± 0.0 ^a	5.8 ± 0.4 ^b	6.2 ± 0.1 ^b	5.9 ± 0.2 ^b
	3	<1.85	9.6 ± 0.0 ^a	8.0 ± 0.0 ^b	5.9 ± 0.3 ^c	6.1 ± 0.1 ^c	8.6 ± 0.0 ^d
	7	<1.85	10.2 ± 0.7 ^a	9.9 ± 0.7 ^a	5.3 ± 0.0 ^b	5.7 ± 0.1 ^c	8.7 ± 0.1 ^d
	14	<1.85	9.0 ± 0.0 ^a	7.6 ± 0.0 ^b	5.0 ± 0.1 ^c	5.0 ± 0.1 ^c	8.3 ± 0.0 ^d
	21	<1.85	8.6 ± 0.0 ^a	7.0 ± 0.0 ^b	4.7 ± 0.1 ^c	4.9 ± 0.2 ^c	8.1 ± 0.0 ^d
CB (log cfu/g)	0	<1.85	<1.85	<1.85	<1.85	<1.85	<1.85
	1	<1.85	4.6 ± 0.0 ^a	3.5 ± 0.0 ^b	<1.85	<1.85	<1.85
	3	<1.85	<1.85	<1.85	<1.85	<1.85	5.8 ± 0.0
	7	<1.85	<1.85	<1.85	<1.85	<1.85	5.8 ± 0.1
	14	<1.85	<1.85	<1.85	<1.85	<1.85	5.4 ± 0.1
	21	<1.85	<1.85	<1.85	<1.85	<1.85	5.4 ± 0.2
YM (log cfu/g)	0	<2.85	<2.85	<2.85	<2.85	3.2 ± 0.1	<2.85
	1	<2.85	<2.85	<2.85	<2.85	3.2 ± 0.1	<2.85
	3	<2.85	<2.85	<2.85	<2.85	<2.85	6.8 ± 0.7
	7	<2.85	<2.85	<2.85	<2.85	<2.85	6.9 ± 0.3
	14	<2.85	<2.85	<2.85	<2.85	3.8 ± 0.4 ^a	7.9 ± 0.6 ^b
	21	<2.85	<2.85	<2.85	<2.85	3.5 ± 0.2 ^a	7.7 ± 0.9 ^b

Means ± SD (n = four jars). In the same line, values with different superscripts are significantly ($P < 0.05$) different.

A, acidified silage; F0, fermented silage, with numbers indicating salt levels, with a: standard, b: pH adjusted to 5.1 and c: added propionic acid; LAB, lactic acid bacteria; CB, coliform bacteria; YM, yeasts and molds; Nd, not determined.

(F2c) had no advantages compared to F2a; in contrast, the levels of yeasts and molds slightly increased in this silage. Finally, the addition of 40 g/kg salt (F4) did not offer any advantages compared to F2a or F0, as acidification was slower and the high levels of coliform bacteria and yeasts and molds indicate microbiological instability. This indicates that the occurring coliform bacteria are not very sensitive to salt, and thus, their disappearance in F2a must have resulted mainly from acidification. No aerobic spores were detected in any of the silages, not even in those that did not reach the pH values <4.5, low enough for preservation.

Effect of Silage Treatments on Nitrogenous Compounds

Levels of NPN shown in Table 4 result from production (proteolysis or autolysis [Raa and Gildberg 1982]) and consumption (microbial assimilation). In silage A, in which microbiological activity is negligible, we therefore

TABLE 4.
PHYSICOCHEMICAL CHANGES DURING EXPERIMENTAL SILAGES AT 37°C OF
BLUE-JACK MACKEREL WITH *LACTOBACILLUS PLANTARUM* LU853

	Period (d)	Treatment					
		A	F0	F2a	F2b	F2c	F4
pH	0	3.8	6.7	6.6	5.1	6.5	6.6
	1	3.6	5.4	4.8	4.8	5.0	6.0
	2	3.8	4.4	3.8	3.7	4.2	4.6
	3	3.7	3.9	3.6	3.7	3.7	4.0
	7	3.8	3.9	3.9	3.8	4.0	4.0
	14	3.8	4.1	3.9	3.8	4.0	4.0
	21	3.8	4.1	3.9	4.0	4.0	4.1
NPN (g/100 g N)	0	24 ± 1 ^a	12 ± 1 ^{bc}	13 ± 0 ^b	17 ± 1 ^d	17 ± 0 ^d	11 ± 0 ^c
	1	Nd	Nd	Nd	Nd	Nd	Nd
	2	Nd	Nd	Nd	Nd	Nd	Nd
	3	45 ± 5 ^a	58 ± 2 ^b	67 ± 2 ^c	68 ± 2 ^c	63 ± 6 ^c	47 ± 1 ^a
	7	69 ± 1 ^a	62 ± 1 ^b	62 ± 2 ^b	67 ± 4 ^b	78 ± 2 ^c	53 ± 1 ^d
	14	Nd	Nd	Nd	Nd	Nd	Nd
	21	83 ± 5 ^a	60 ± 4 ^b	59 ± 1 ^b	70 ± 1 ^c	71 ± 3 ^c	46 ± 5 ^d
TVBN (mg/g N)	0	14 ± 1 ^a	9 ± 1 ^b	9 ± 1 ^b	10 ± 1 ^b	12 ± 1 ^a	8 ± 1 ^b
	1	Nd	Nd	Nd	Nd	Nd	Nd
	2	Nd	Nd	Nd	Nd	Nd	Nd
	3	12 ± 0 ^a	56 ± 3 ^b	50 ± 2 ^c	46 ± 2 ^c	72 ± 5 ^d	47 ± 2 ^c
	7	15 ± 2 ^a	58 ± 4 ^b	51 ± 1 ^c	51 ± 3 ^{bc}	80 ± 5 ^d	53 ± 1 ^{bc}
	14	16 ± 1 ^a	62 ± 0 ^b	52 ± 2 ^{cc}	51 ± 1 ^c	75 ± 7 ^d	56 ± 2 ^e
	21	18 ± 2 ^a	61 ± 2 ^b	53 ± 1 ^c	54 ± 2 ^c	73 ± 2 ^d	53 ± 5 ^c
SMR (g/100 g)	0	49.9 ± 0.4 ^a	21.7 ± 0.9 ^b	0.1 ± 0.0 ^c	60.4 ± 0.3 ^d	0.1 ± 0.0 ^c	0.0 ± 0.0 ^e
	1	Nd	Nd	Nd	Nd	Nd	Nd
	2	Nd	Nd	Nd	Nd	Nd	Nd
	3	58.6 ± 0.3 ^a	65.2 ± 0.9 ^b	62.6 ± 0.8 ^c	77.0 ± 0.4 ^d	74.1 ± 0.8 ^e	58.8 ± 0.9 ^a
	7	62.8 ± 0.4 ^a	65.1 ± 0.6 ^b	62.4 ± 0.9 ^a	76.7 ± 0.3 ^c	72.6 ± 0.9 ^d	60.7 ± 0.7 ^e
	14	68.7 ± 0.7 ^a	65.5 ± 0.9 ^b	62.5 ± 0.3 ^c	77.5 ± 0.4 ^d	74.4 ± 1.2 ^e	60.4 ± 0.7 ^f
	21	71.4 ± 0.5 ^a	66.2 ± 0.8 ^b	63.9 ± 0.7 ^c	76.8 ± 0.3 ^d	74.6 ± 0.5 ^e	60.2 ± 0.8 ^f

Means ± SD (*n* = four jars). In the same line, values with different superscripts are significantly (*P* < 0.05) different.

A, acidified silage; F0, fermented silage, with numbers indicating salt levels, with a: standard, b: pH adjusted to 5.1 and c: added propionic acid; NPN, non-protein nitrogen; TVBN, total volatile basic nitrogen; SMR, silage moisture release; Nd, not determined.

assume that consumption of NPN is very low. Apparently, proteolytic activity from ground fish released approximately 80 g NPN/100 g N; this indicates that 80% of crude protein is degraded into peptides and smaller compounds. In the fermented silages, NPN levels were lower than in silage A. This indicates that the microflora does not increase the extent of proteolysis, but rather assimilates some NPN.

Considering the formation of TVBN, an interesting difference between the acidified (A) and fermented silages can be observed. Whereas in silage A the level of TVBN remains stable at 15–19 mg/g N (Table 4), the fermented silages show significantly higher levels ranging from 52–75 mg TVBN. Although no TVBN limit for acceptability of *Trachurus* has been established (Gimenez *et al.* 2002), acceptable levels for whole and filleted fish range from 25–40 mg/100 g flesh (Gimenez *et al.* 2002; Chytiri *et al.* 2004), which corresponds to 15–25 mg/g N in fatty fish such as mackerel. Whereas the acidified silage complies with this level, the fermented silages clearly exceed this, which we relate to the effect of microbial activity (Chytiri *et al.* 2004). All the same, the fermented silages were free of objectionable odors.

Effect of Silage Treatments on Water-binding Properties

The water-binding properties affect the consistency of the silage and the amounts of moisture released. In Figs. 1 and 2, the available data of SMR were plotted against their corresponding pH and NPN levels, respectively. As already known, pH is a major factor determining the water-binding properties of muscle proteins, hence directly affecting the SMR of fresh silages (from our data, a correlation coefficient -0.919 was obtained). Once proteins gradually break down, NPN values will increase and SMR levels will rise (as illustrated in Fig. 2). In addition, the small range (50–80) of SMR levels at a relatively large range (20–80) of NPN values indicates that other additional factors have

FIG. 1. PLOT OF pH AGAINST SILAGE MOISTURE RELEASE (SMR) (30-DATA POINTS)

FIG. 2. PLOT OF NON-PROTEIN NITROGEN (NPN) AGAINST SILAGE MOISTURE RELEASE (SMR) (24-DATA POINTS)

a contributing effect. One of these factors is very likely the salt content, as it has been observed elsewhere (Wheaton and Lawson 1985; Lin and Park 1998) that salt at the levels applied here increases the water-binding properties of fish muscle.

CONCLUSIONS

Considering the criteria for stable, high-quality fish silage, it can be concluded that fermentation at 17°C is unsuitable because of slow acidification; this implies that at “ambient” temperatures of the Azores (approximately 17–20°C), the addition of acids will be required to shorten the critical period. Other unsuitable options (fermentations studied at 37°C) were the addition of 40 g/kg salt (F4) and the combination 20 g/kg salt + propionate (F2c) because of the resulting microbiological instability. Rapid acidification and microbiologically stable silages were obtained by fermentation with *Lb. plantarum* LU853, either (F2a) or not (F0) in combination with 20 g/kg salt addition; F2a acidified more rapidly, which may be an advantage for its microbiological stability. Proteolysis resulting in NPN was similar in all silages; however, in acidified silages, the levels of TVBN were significantly lower. The final choice of silage-making method would therefore depend on the incubation temperature and on the importance given to the minor drawbacks attached to each of the scenarios, namely, the environmental impact of adding acids (silage A), the environmental impact of adding salt, and the generation of higher TVBN (silage F2b) or of slightly less rapid acidification, and generating higher TVBN (silage F0). For practical purposes, we expect that acidification, such as in silage A, will be the most feasible option.

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REFERENCES

- ADAMS, M.R., COOKE, R.D. and TWIDDY, D.R. 1987. Fermentation parameters involved in the production of lactic acid preserved fish-glucose substrates. *Int. J. Food Sci. Technol.* 22, 105–114.
- AHMED, J. and MAHENDRAKAR, N.S. 1996. Chemical and microbiological changes in fish viscera during fermentation ensiling at different temperatures. *Bioresour. Technol.* 59, 45–46.
- ANONYMOUS. 1988. *Pescado. Determinação do teor de azoto básico volátil (A.B.V.T.). Método de Conway. Norma Portuguesa NP2930*, Instituto Português de Qualidade, Lisbon, Portugal.
- AOAC. 1990. *Official Methods of Analysis*, 15th Ed., Association of Official Analytical Chemists, Washington, DC.
- BATISTA, I., MENDES, R. and NUNES, M.C. 1987. Ensilados ácidos e biológicos: preparação e estudo das alterações químicas ocorridas durante a armazenagem. In *Textos das Comunicações do Seminário sobre Aquacultura*, ICBAS/Associação Portuguesa de Recursos Hídricos, April 9–11, Oporto, Portugal.
- BONESTROO, M.H., KUSTERS, B.J., DE WIT, J.C. and ROMBOUTS, F.M. 1992. Glucose and sucrose fermenting capacity of homofermentative lactic acid bacteria used as starters in fermented salads. *Int. J. Food Microbiol.* 15, 365–376.
- CHYTIRI, S., CHOULIARA, I., SAVVAIDIS, I.N. and KONTOMINAS, M.G. 2004. Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout. *Food Microbiol.* 21, 157–165.
- DAPKEVICIUS, M.L.N.E., BATISTA, I., NOUT, M.J.R., ROMBOUTS, F.M. and HOUBEN, J.H. 1998. Lipid and protein changes during the ensilage of blue whiting (*Micromesistius poutassou* Risso) by acid and biological methods. *Food Chem.* 63, 97–102.
- DONG, F.M., FAIRGRIEVE, W.T., SKONBERG, D.I. and RASCO, B.A. 1993. Preparation and nutrient analysis of lactic acid bacterial ensiled salmon viscera. *Aquaculture Res.* 109, 351–366.
- GIMENEZ, B., RONCALES, P. and BELTRAN, J.A. 2002. Modified atmosphere packaging of filleted rainbow trout. *J. Sci. Food Agric.* 82, 1154–1159.

- JACKSON, T.C., ACUFF, G.R. and DICKSON, J.S. 1997. Meat, poultry and seafood. In *Food Microbiology. Fundamentals and Frontiers*, (M.P. Doyle, L.R. Beuchat and T.J. Montville, eds.) pp. 83–100, ASM Press, Washington, DC.
- JOHNSEN, F. and SKREDE, A. 1981. Evaluation of fish viscera silage as a feed resource. *Acta Agric. Scand.* 31, 22–28.
- KOUTSOUMANIS, K.P., KENDALL, P.A. and SOFOS, J.N. 2003. Effect of food processing-related stresses on acid tolerance of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 69, 7514–7516.
- LASSEN, T.M. 1995a. Biological quality of fermented fish offal and chicken by-products. *Agric. Sci. Finland* 4, 27–33.
- LASSEN, T.M. 1995b. Evaluation of conditions for fermentation of fish offal. *Agric. Sci. Finland* 4, 11–17.
- LIN, T.M. and PARK, J.W. 1998. Solubility of salmon myosin as affected by conformational changes at various ionic strengths and pH. *J. Food Sci.* 63, 215–218.
- LINDGREN, S. and PLEJE, M. 1983. Silage fermentation of fish or fish waste products with lactic acid bacteria. *J. Sci. Food Agric.* 34, 1057–1067.
- MACKIE, I.M., HARDY, R. and HOBBS, G. 1971. *Fermented Fish Products*. FAO Fisheries Reports, No. 100, Food and Agriculture Organization of the United Nations, Rome, Italy.
- NOUT, M.J.R., DE DREU, M.A., ZUURBIER, A.M. and BONANTS-VAN LAARHOVEN, T.M.G. 1987. Ecology of controlled soyabean acidification for tempe manufacture. *Food Microbiol.* 4, 165–172.
- NOUT, M.J.R., ROMBOUTS, F.M. and HAVELAAR, A. 1989. Effect of accelerated natural lactic fermentation of infant food ingredients on some pathogenic micro-organisms. *Int. J. Food Microbiol.* 8, 351–361.
- OWENS, J.D. and MENDOZA, L.S. 1985. Enzymatically hydrolysed and bacterially fermented fishery products. *J. Food Technol.* 20, 237–293.
- RAA, J. and GILDBERG, A. 1982. Fish silage: A review. *CRC Crit. Rev. Food Sci. Nutr.* 16, 383–419.
- RAA, J., GILDBERG, A. and STROM, T. 1983. *Valorizing Waste for Feeds and Food*, (D.A. Ledward, A.J. Taylor and R.A. Lawrie, eds.) pp. 117–132, Butterworths, London, U.K.
- SCHABOLT, C., ROSS, T. and MCMEEKIN, T.A. 2001. Differentiation of the effects of lethal pH and water activity: Food safety implications. *Lett. Appl. Microbiol.* 32, 99–102.
- SNEDECOR, G.W. and COCHRAN, W.G. 1980. *Statistical Methods*, 7th Ed., Iowa State University Press, Ames, IA.
- STROM, T. and EGGUM, B.O. 1981. Nutritional value of fish viscera silage. *J. Sci. Food Agric.* 32, 115–120.

- SYNNES, O.M. and OPSTAD, S. 1995. Effect of fish silage on the yield and chemical composition of Italian ryegrass (*Lolium multiflorum* Lam.). *Acta Agric. Scand. B Soil Plant Sci.* 45, 68–72.
- WHEATON, F.W. and LAWSON, T.B. 1985. Other preservation methods. In *Processing Aquatic Food Products*, (F.W. Wheaton and T.B. Lawson, eds.) pp. 274–275, John Wiley & Sons, New York, NY.