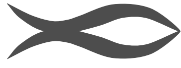

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RESEARCH PAPER

Biotransformation of Seafood Processing Wastes Fermented with Natural Lactic Acid Bacteria; The Quality of Fermented Products and Their Use in Animal Feeding

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Abstract

Lactic acid bacteria species naturally present in fish (*Streptococcus* spp., *Lactobacillus brevis*, *Lactobacillus plantarum, Pediococcus acidilactici* and *Enterococcus gallinarum*) and formic acid were compared for production of fish silage, which are protein hydrolysates with immune stimulating properties. It is the first report for *Enterococcus gallinarum* to be used for producing fermented products. In this study, chemical and microbiological qualities of fish silage by acid or fermented methods were assessed after ripening of silages. It was observed that ripening was completed in maximum two weeks for all silage groups. Then, acid and fermented fish silage were spray dried and analysed for chemical and nutritional properties. As results of the study, these bacteria can be used as starter cultures in fermented products, especially for fish silage. In respect to essential/nonessential amino acid ratio (E/NE), the best groups among the spray-dried fish silages were prepared with formic acid, *Lb. plantarum* and *Pd. acidilactici*, respectively. Total antioxidant activity (TAO) of spray-dried fish silage was compared with ascorbic acid and TAO of spray-dried fish silages was found in range of 1.92 - 2.86 mg AA/g. The highest DPPH (diphenylpicrylhydrazyl) radical-scavenging ability of spray-dried fish silages was detected in AC group produced with *Pediococcus acidilactici* (20.26%) and GL group produced with *Enterococcus gallinarum* (16.95%), but there were no significant differences observed in other groups. According to the in-vitro gas production assessment, spray-dried fish silages generally had considerably high rate of digestibility. It was determined that the acid and fermented fish silage powders had high digestibility and valuable feed sources according to the results of the proximate analysis, amino acids compositions, total antioxidants, DPPH inhibition rates and in-vitro digestibility assessments.

Keywords: Fermented fish silage, lactic acid bacteria, chemical quality, antioxidant and antimicrobial activity, in-vitro gas production.

Introduction

Fish wastes management is a growing concern in seafood processing industry. According to EC regulation (1774/2002), by-products from fish processing plants for human consumption are considered as Category 3 materials. It was reported by European authorities that category 3 materials can be processed for human and animal consumption in a way to contribute to the environment and public health. They are used for the production fish meal, but this technique needs high energy, serious investment and labour costs. As a low cost alternative, biotechnological methods such as controlled fermentation are gaining importance to recover biomolecules from seafood processing wastes.

Fish silage is traditionally produced by mixing minced fish mass and acid. Fermentation using lactic acid bacteria has a better impact than acid method as it provides beneficial effects such as antibacterial activity and also prevents lipid oxidation of silage during ripening (Raa & Gilberg 1982). These microorganisms produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins (Yusuf & Hamid 2013). These compounds have an important effect on the inhibition of the spoilage microorganism, and also a desired effect on the taste and texture of the foods. In order to benefit from fermentation the selection of commercially applicable lactic acid bacteria strains is of great importance for feed and food industry. The selection and comparison of lactic acid bacteria strains from fish flora may provide the definition of the most appropriate types for the production of fish silage (Ozyurt, 2016).

The share of livestock in the agricultural production is around 32.5% in Italy, 33% in Turkey, 38.8% in Spain and 40% in the Netherlands and France (Akman, 2013). The share of total energy and protein consumption in animal products are 17.0%

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and 38.4% in worldwide, respectively. Animal nutrition is one of the main limiting factors in animal production, especially in ruminants leading to proteins being the main restrictive components due to the limited presence and high cost of protein sources. Feed obtained from seafood waste has the potential usage as high protein supplements for farm animals. In several recent studies, antibacterial and antioxidant activities of lactic acid bacteria have been determined in fish silage (Sachindra & Bhaskar, 2008; Rai *et al*., 2009; Murthy, Rai, & Bhaskar, 2014). Van Hylckama Vlieg and Hugenholtz (2007) reported that when the lactic acid bacteria used as a starter culture for fermentation, these bacteria produced some organic acids with preservative and nutritional effect. It was also reported that fish silage was well accepted by the animal husbandry and gave comparable results with the commercial feed (Jangaard, 1987). Acceptable data were also obtained when fish silage was incorporated in ruminant feeds. Perez (1995) recorded that smaller quantities of fish silage could be used for the supplication of the necessary amino acids such as methionine and lysine for lactating dairy cows. Salas, Gutiérrez, Juárez, Flores, and Perea (2011) proposed the usage of the devil fish (*Pterigoplychthys* spp.) in silage for consumption of pigs, sheep and cattle species that are highly accepted for human consumption. Tejeda-Arroya *et al*. (2015) recorded that the inclusion of up to 18% of fish silage in the lamb diets might improve the productive performance and the ruminal fermentation kinetics without any negative effects. Some work have been done recently on fish silage (Tanuja, Mohanty, Kumar, Moharana, & Nayak, 2014; Nørgaard, Petersen, Tørring, Jørgensen, & Lærke, 2015; Ozyurt, Gökdoğan, Şimşek, Yuvka, Ergüven, & Kuley, 2016; Goosen, Wet, & Görgens, 2016), but limited research has been directed to the potential of using spray-dried fish silage in feed formulations. The aim of this study was to compare acid silage with fermented silages, which were produced by using lactic acid bacteria (LAB) naturally existing in fish, for biotransformation of the seafood processing wastes into silages. Therefore, five LAB strains (*Streptococcus* spp., *Lb. brevis*, *Lb. plantarum, Pd. acidilactici* and *Ent. gallinarum*) which were isolated from natural fish flora according to the PCR result in our previous study (Ozyurt *et al*., 2016) were selected as starter cultures for the production of fish silage fermentation.

Materials and Methods

Total aerobic and anaerobic microorganism counts, lactic acid bacteria, fungi, total coliform, patogen bacteria (*E. coli, Salmonella, Staphylococcus aureus, Listeria* spp.*)*, pH, thiobarbituric acid-reactive substances (TBARs) and non-protein nitrogen analyses (NPN) were carried out on acid and fermented fish silages during maturation process at the start of this study. Then proximate compositions, amino acid compositions, antioxidant and antimicrobial activities of spray-dried acid and fermented fish silage were identified and the possibilities of their utilization in livestock as feed materials were investigated with in-vitro gas production techniques.

Bacterial Strains

A total of eight LAB strains (*Enterococcus gallinarum, Streptococcus spp, Lactobacillus lactis* subsp. *lactis, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus acidilactici, Leuconostoc mesenteroides* subsp. *cremoris* and *Lactobacillus delbrueckii* subsp. *lactis*) were isolated from the natural fish flora (skin, muscle and gut of sea bream, mullet, sea bass, carp and European catfish) according to the PCR results in our previous study (Ozyurt *et al*., 2016). Five LAB strains (*Streptococcus* spp., *Lb. brevis*, *Lb. plantarum, Pd. acidilactici* and *Ent. gallinarum*) were selected as starter cultures for the production of fish silage fermentation according to the previous study results.

Silage Preparation and Drying Conditions

Six groups of silage trials which were acid (formic acid) and five bacteria (*Streptococcus* spp., *Lb. brevis*, *Lb. plantarum, Pd. acidilactici* and *Ent. gallinarum*) were carried out with the raw material of sea bass processing waste. Sea bass (*Dicentrarchus labrax*) processing wastes comprising of head, skin, frame and gut were collected from seafood processing plants located in Adana (Turkey) and transported in ice to the laboratory. Fish wastes were minced by a grinder and divided into six equal groups. Group 1 (control: C) included formic acid (3%) without any LAB inoculation. Group 2, 3, 4, 5 and 6 were inoculated with *Lb. plantarum* (LP), *Pd. acidilactici* (AC), *Ent. gallinarum* (EG), *Lb. brevis* (LB) and *Streptococcus* spp. (ST) respectively. After mincing, 15% molasses as a fermentable carbohydrate source were added to the mixture (Shirai *et al*., 2001) and the mixture were inoculated with LAB strains (5%, 10^8 cfu/ml). Then, 250 mg/kg butylated hydroxytoluene (BHT) and 2.2 g/kg potassium sorbate were added as an antioxidant and fungicide in all silage groups, respectively. Each group was conducted as triplicates and stored at room temperature (27-28°C) in plastic jars with caps and stirred daily until ripening.

Total aerobic and anaerobic microorganism counts, lactic acid bacteria, fungi, total coliform, pathogen bacteria (*E. coli*, *Salmonella*, *Staphylococcus aureus* and *Listeria* spp*)*, pH, TBARs and NPN analysis were carried out on a regular base (1st, 2nd and 3rd week) until acid and fermented silage processes occurred. After the maturation process, in order to increase the shelf life of silages and to use them for other purposes, their lipid contents were extracted according to the method of Crexi, Souza-

Soares, & Pinto, (2009). After this process, all silage samples were spray dried using a Buchi Mini Spray Dryer (B-290, Switzerland). Before drying, the fish silages were mixed with maltodextrin (DE:18-20) in proportion of 1:1 (w/w, on dry basis) for an efficient drying. The solutions were constantly stirred during the drying process. The inlet and outlet temperatures were maintained at 160°C and 90°C, respectively. The aspiration rate was about 30 m^3/h and the feeding rate was 20 ml/min. The powders obtained were kept at 4°C until analysed. As a result, spray-dried silages were analysed in terms of proximate compositions, amino acid compositions, antimicrobial and antioxidant activities and *in-vitro* gas production.

Microbiological Analyses of Fish Silages

Triplicate samples were taken to estimate the total viable counts (TVC) from each silage groups. Silage samples (10 g) were mixed with 90 ml of sterile Ringer solution (1/4 strength) and then stomached for 3 min. Further decimal dilutions were made, and then, 0.1 ml of each dilution was pipetted onto the surface of plate count agar (Fluka 70152, Steinheim, Switzerland) plates in triplicate. Then, plates were incubated for 2 days at 30°C. Total anaerobic bacteria was counted in plate count agar (Merck 1.05463) in anaerobic jars and incubated for 4 days at 20°C. Molds and yeast counts were evaluated using Potato Dextrose Agar (Oxoid, CM0139) and incubated for 5 days at 25°C. For total *Enterobacteriaceae* and lactic acid bacteria, violet red bile agar (VRBA, Merck 1.01406) and MRS agar (Merck 1.10660) were used. One ml aliquots of the each dilution were transferred to petri dishes using the pour plate method. They were incubated for 5 days (MRS agar) and 24 h (VRBA) at 30 °C.

Tryptone Bile Agar with X-Glucuronide (Oxoid, CM0945), a chromogenic medium was used for the detection of *E. coli*. Plates were incubated for 18-24 hr at 37 °C. Baird-Parker agar (Merck, 1.10675) was used for the *Staphylococcus aureus* counts. Plates were incubated at 37°C for 2 days. XLD agar (Merck, 1.05287) was used for the *Salmonella* counts. Plates were incubated at 37°C for 24 hours (ISO 6579:2002 Method). API 20E (BioMereux, France) was used to confirm bacterial isolates. *Listeria* spp. was enumerated according to the method of ISO 11290-1:1996 using Palcam and Oxford Agar at 37°C for 24 hours. For confirmation of Listeria spp., API Listeria kit (BioMerieux, La Balme-les-Grottes, France) was used. The inoculated strip was incubated for 16-24h and the colour reactions were noted as either positive or negative. The gathered results were analysed using the APILAB PLUS software (Biomerieux, France).

Chemical Analyses of Fish Silages

pH was measured using a digital pH metre (WTW 315i, Germany). Non-protein nitrogen (NPN) analysis was used to estimate the protein autolysis. Samples (40 g) were stirred with TCA (60 ml, 20% thrichloracetic acid) and filtered. Then, the nitrogen content of filtrate was detected with Kjeldahl's procedure and expressed as NPN (AOAC 2002). Thiobarbituric acid-reactive substances (TBARs) were determined according to Tarladgis *et al.* (1960) to evaluate the oxidation stability in acid and fermented silages, and results were expressed as TBARs value, mg MDA (malondialdehyde) eq./ kg silages.

Proximate and Amino Acid Composition of Spray-Dried Fish Silages

Moisture content and crude ash of acid and fermented fish silages were detected in an oven at 103°C and 550°C respectively until the weight of samples became constant. Crude proteins of silages were determined as described in AOAC (1999, 981.10) and crude lipid contents were measured using the procedure of Bligh and Dyer (1959). Amino acid compositions of acid and fermented fish silages were determined by the MAM (Food Institute of Marmara Research Centre), TUBITAK (Scientific and Technological Research Council of Turkey). A Shimadzu 20 Series UFLC (Ultra Fast Liquid Chromatography) with UV detection was used. The method was adapted from literature and modified by TUBITAK MAM (Dimova, 2003).

Antioxidant Activity of Spray-Dried Fish Silages

In-vitro antioxidant activities of spray-dried fish silages were evaluated with the analyses of both total antioxidant activity (TAO) and scavenging of DPPH (diphenylpicrylhydrazyl). Spray-dried fish silage (1 g) was mixed with distilled water (100 ml) and the mixture was stirred at room temperature for 30 min. Then, the mixture was centrifuged at 3000 g for 10 min and then the supernatant was used for further analyses. TAO of the spray-dried fish silages was determined by the phosphomolybdenum method of Prieto, Pineda, & Aguilar (1999). The sample (0.3 ml) was mixed with 3 ml of reagent solution [3.0 ml; 0.6 M sulphuric acid: 28 mM sodium phosphate: 4 mM ammonium molybdate (1:1:1 v/v/v)]. Reaction mixture was incubated in a water bath at 95°C for 90 min. After the samples cooled to room temperature, the absorbance was measured at 695 nm. TAO was expressed as µg of ascorbic acid equivalents (AAE) per mg of protein in spray-dried fish silage sample.

The scavenging effect of spray-dried fish silages on DPPH was analysed by using the method of Wu, Chen, & Shiau, (2003) with some minor modifications. Sample solutions were mixed with 1.5 ml of 0.1 mM DPPH in 95% ethanol. Then, the mixture was left for 30 min at room temperature, and the absorbance of the resulting solution was detected at 517 nm. A lower absorbance represented a higher

DPPH scavenging activity. The scavenging effect on DPPH free radical was calculated by using the following equation:

Percentage inhibition (I%) : ((Blank absorbance−Sample absorbance)/Blank absorbance)×100%.

Antimicrobial Activity of Spray-Dried Fish Silages

Antimicrobial activity of silage was evaluated using the disc diffusion method (Murray *et al.* 1995) with minor modifications. The reference strains used in antimicrobial tests were *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC700603, *Enterococus faecalis* ATCC29212, *Pseudomonas aeruginosa* ATCC27853, *Camplylobacter jejuni* ATCC 33560**,** Salmonella Parathypi A NCTC13 and *Yersinia enterocolitica* NCTC 11175. Nutrient agar (Merck 1.05450) was used as the standard test medium for bacteria. The agar plate was spread with the inoculum containing 10^8 cfu/ml pathogenic bacteria. Fifty µL of undiluted spray-dried fish silage (50 mg/ml) were pipetted on sterile filter paper discs (diameter 6 mm), which were allowed to dry in an open sterile petri dish in a biological safety cabinet with vertical laminar flow. Paper discs were placed on the inoculated agar surfaces. After incubation at 37±1°C for 18–24 h for bacteria, diameters (mm) of the zones of bacterial inhibition minus the disc diameter were recorded. Each test was performed in triplicate and the results were analysed for statistical significance. Antibiotics with positive responses were used as controls for the plates. Tetracycline and Neomycine served as positive controls on bacteria.

The determination of the minimum inhibitory concentration (MIC) was carried out as described by the Clinical and Laboratory Standards Institute (2008). One µL of Muller Hinton Broth (MHB) was added in each test tube. One ml of spray-dried fish silage (with stock solution of 50 mg/ml) was added to the first tube in each series (after removing the same volume of broth) and a serial dilution technique was used to find the minimal concentration of fish silage. Bacterial suspension was adjusted to match the 0.5 McFarland turbidity standard (10^6 cfu/ml). The inoculum suspension (1 ml) of each bacterial strain was then added to each tube containing silage powder. The final concentrations of the silage powder were 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19 mg/ml. The tubes were incubated at 35°C for 18–24 hours after which the MIC was recorded as the lowest concentration producing no visible growth (absence of turbidity and/or precipitation) as observed through naked eye.

In-Vitro Gas Production of Spray-Dried Fish Silages

The study conducted in Cukurova University, Faculty of Agriculture Research Farm. All measurements were based on 100% dry matter in triplicate. Spray-dried fish silages were incubated *in vitro* with rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). Rumen fluid was obtained from an eight year old and approximately 550 kg body weight of one fistulated Holstein infertile cow after one week adjustment period of a diet. Fistulated Holstein infertile cow fed twice daily with a diet containing rough feed (60%) and concentrate (40%). Basal diet (corn silage and concentration feed) was included 2500 kcal/kg metabolizable energy and with a content of 16% crude protein. Rumen fluid was collected before morning feeding, squeezed through four layers of cheesecloth and flushed with CO_2. Then the rumen fluid was added to a buffered mineral solution in the ratio of 1:2. Approximately 0.200 g dry weight of spray-dried fish silages samples were weighed in triplicate into calibrated glass syringes of 100 ml. The syringes were pre-warmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture into each syringe followed by the incubation in a water bath at 39°C.

Gas production was recorded at 3, 6, 12, 24, 48, 72 and 96 h after incubation and corrected with blank incubation. Cumulative gas production data of fish silages were fitted to non-linear exponential model as: $Y = a+b\,(1-e^{-ct})$ (Orskov & McDonald 1979).

Where "Y" was gas production at time t. "a" was the gas production from the immediately soluble fraction (ml). "b" was the gas production from the insoluble fraction (ml). "c" was the gas production rate constant (h^{-1}) and "t" was the incubation time (h). "a+b" was the potential gas production (mL).

Metabolizable energy (ME, MJ/kg DM) values of spray-dried fish silages were estimated as follows (Menke & Steingass 1988):

$$ME\,(MJ/kg\,DM) = 1.06+0.157GP+0.084CP+0.22EE-0.081CA$$

where, "GP" was 24 h net gas production (mL/200 mg DM), "CP" was crude protein (%), "EE" was ether extract (%, at 100% dry matter), "CA" was crude ash content (%, at 100% dry matter).

Organic matter digestibility (OMD, %) values of spray-dried fish silages were calculated using the equation of Close and Menke (1986) as follows:

$$OMD,\% = 0.7602GP+0.6365CP+22.53$$

where "CP" was crude protein (%), "GP" was net gas production (ml/200 mg DM) at 24 h of incubation.

Net energy for lactation (NE_L, MJ/kg DM) values of spray-dried fish silages were calculated using the equation of Menke and Steingass (1988) as follows:

$$NE_L(MJ/kg\,DM) = 0.075GP + 0.087CP + 0.161EE + 0.056NFE - 2.422$$

where, "GP" was 24 h net gas production (mL/200 mg DM), "CP" was crude protein (%), "EE" was ether extract (%, at 100% dry matter), "NFE" was nitrogen free extract (%).

Statistical analyses

Differences between means were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test.

Results and Discussion

Chemical Assessment of Fish Silages

Acid and fermented fish silages were stirred regularly every day for two weeks and their pH values were measured daily. The changes of pH values of acid and fermented fish silages produced by sea bass wastes are given in Table 1. The initial pH value for the acid silage was recorded as 3.42, while it was measured as 6.11 to 6.27 in fermented fish silages. pH showed a more rapid decrease in acid silage after the addition of formic acid, while the pH of fermented silages decreased gradually. After two weeks, the recommended pH values (pH 4.5) for fish silages were reached in fermented ones and the values also remained stable under this value after ripening for a week. Fish or fish wastes contain slight amounts of fermentable sugar. Therefore, an additional energy source must be added for fermentation to fish silages for lactic acid bacterial growth. In our case, molasses were added in order to provide this. The decrease in pH in fermented silages showed a prominent utilization of the energy source, the occurrence of organic acids and subsequently the inhibition of the growth of spoilage microorganism were also observed (Ozyurt, 2016).

NPN contents which express the protein solubilisation were measured for three weeks and showed in Table 2. NPN values show the protein degradation which was caused by the release of free amino acids and short chained peptides. The degree of this hydrolysis can change depending on the factors such as temperature, storage time and the structure of the raw material. It was reported that NPN contents of silages were increased as the storage time and temperature increased (Özyurt *et al*., 2016; Bhaskar & Mahendrakar, 2007). In this study, the initial NPN value of raw fish wastes was 0.09±0.00 g/100g after the silage production and the values in all silage groups showed significant increases during the three week trial period (P<0.05). Although the lowest NPN value was observed in the AC group (0.39 g/100g) in the first week (P<0.05), there were no significant differences among the groups (P>0.05) at the second and third weeks (0.40-0.48 g/100g and 0.45-0.54 g/100g, respectively). Some researchers observed that ensiling by acid caused higher protein solubilisation values compared to fermented ones (El-Ajnaf, 2009). However, such relationship between acid and fermented fish silages was not observed in this study.

Lipid oxidation measurements in acid and fermented silages were analysed by TBARs which quantifies the secondary products of the lipid oxidation. In Table 2, the initial TBARs value of raw sea bass wastes and the changes of TBARs values of acid and fermented silages during a three week period can be seen. The initial TBARs value of raw fish wastes was detected as 0.81 mg MDA eq./kg. In the first week of ripening significant increases in TBARs values were observed in LP, AC and BR groups which were fermented with *Lb. plantarum*, *Pd. acidilactici* and *Lb. brevis,* respectively (P<0.05). TBARs values of FA, GL and ST showed no significant differences to raw material in the first week (P>0.05). During storage, although some fluctuations in TBARs values of all silage groups

Table 1. Changes of pH values in acid and fermented fish silages

Days	FA	LP	AC	GL	BR	ST
0.	3.42±0.02^{aA}	6.27±0.01^{kD}	6.11±0.01^{kB}	6.17±0.04^{lBC}	6.22±0.05^{kCD}	6.27±0.03^{jD}
1.	3.65±0.01^{cA}	5.79±0.04^{jD}	5.46±0.01^{jB}	5.80±0.05^{kD}	5.81±0.03^{jD}	5.57±0.04^{iC}
2.	3.67±0.00^{cA}	5.11±0.01^{iC}	5.11±0.01^{iC}	5.04±0.02^{jB}	5.09±0.00^{iC}	5.06±0.00^{hB}
3.	3.70±0.00^{dA}	5.01±0.01^{hC}	5.06±0.01^{hE}	4.97±0.02^{iB}	5.01±0.01^{hC}	5.04±0.01^{hD}
4.	3.71±0.01^{dA}	5.01±0.00^{hD}	5.05±0.01^{hE}	4.93±0.01^{ghB}	5.01±0.01^{hD}	4.99±0.01^{gC}
5.	3.76±0.00^{efA}	5.01±0.00^{hC}	5.05±0.01^{ghD}	4.91±0.01^{gB}	5.00±0.00^{hC}	4.90±0.02^{fB}
6.	3.80±0.02^{ghA}	5.00±0.00^{hD}	5.00±0.00^{gD}	4.82±0.01^{eB}	4.92±0.01^{gC}	4.83±0.00^{eB}
7.	3.75±0.00^{eA}	4.83±0.00^{gB}	4.96±0.01^{fE}	4.83±0.00^{eB}	4.91±0.00^{gD}	4.88±0.00^{fC}
8.	3.79±0.01^{fgA}	4.77±0.01^{fB}	4.81±0.08^{eB}	4.94±0.01^{hiC}	4.95±0.02^{gC}	4.91±0.03^{fC}
9.	3.79±0.01^{fgA}	4.65±0.01^{eB}	4.71±0.02^{dC}	4.87±0.02^{fE}	4.80±0.03^{fD}	4.73±0.04^{dC}
10.	3.82±0.01^{hiA}	4.54±0.02^{dB}	4.54±0.02^{cB}	4.73±0.02^{dE}	4.67±0.03^{eD}	4.62±0.03^{cC}
11.	3.85±0.02^{iA}	4.55±0.02^{dC}	4.47±0.02^{bB}	4.60±0.01^{cD}	4.59±0.02^{dD}	4.59±0.01^{cD}
12.	3.61±0.03^{bA}	4.51±0.02^{cCD}	4.46±0.03^{bB}	4.61±0.04^{cE}	4.47±0.02^{cBC}	4.55±0.02^{bD}
15.	3.77±0.04^{efgA}	4.38±0.02^{bB}	4.39±0.06^{aB}	4.46±0.02^{bB}	4.39±0.05^{bB}	4.40±0.06^{aB}
21.	3.75±0.03^{eA}	4.34±0.04^{aB}	4.35±0.03^{aB}	4.40±0.03^{aB}	4.35±0.04^{aB}	4.38±0.05^{aB}

FA: Formic Acid; LP: *Lb. plantarum;* AC: *Pd. acidilactici*; GL: *Ent. gallinarum;* BR: *Lb. brevis* ST: *Streptococcus* spp
The values are expressed as mean ± standard deviation, n=4
Values in a same column followed by different letters (a-l) indicate significant differences of the parameter with respect to the storage time (P<0.05).
Values in a same line followed by different letters (A-E) indicate significant differences of the parameter with respect to the kind of silage (P<0.05).

Table 2. Non-protein nitrogen values (NPN) and Thiobarbituric acid-reactive substances values (TBARs) of acid and fermented fish silages

	Silage Groups	Raw fish wastes	I. Week	II. Week	III. Week
NPN (g/100g)	FA	0.09 ± 0.00^{a}	0.58 ± 0.01^{bBC}	0.48 ± 0.03^{bA}	0.45 ± 0.07^{bA}
	LP		0.61 ± 0.06^{cC}	0.40 ± 0.00^{bA}	0.46 ± 0.03^{bA}
	AC		0.39 ± 0.00^{bA}	0.40 ± 0.03^{bA}	0.54 ± 0.05^{cA}
	GL		0.61 ± 0.02^{dBC}	0.45 ± 0.01^{bA}	0.54 ± 0.01^{cA}
	BR		0.56 ± 0.03^{cBC}	0.43 ± 0.02^{bA}	0.46 ± 0.01^{bA}
	ST		0.50 ± 0.00^{bB}	0.48 ± 0.06^{bA}	0.45 ± 0.02^{bA}
TBARs (mg MDA eq./kg)	FA	0.81 ± 0.02^{a}	1.10 ± 0.01^{aC}	1.03 ± 0.03^{aD}	1.85 ± 0.45^{bB}
	LP		1.11 ± 0.01^{dC}	0.96 ± 0.03^{bC}	1.05 ± 0.03^{cA}
	AC		1.11 ± 0.01^{cC}	0.98 ± 0.00^{bCD}	1.07 ± 0.06^{cA}
	GL		1.03 ± 0.04^{aB}	0.89 ± 0.07^{aB}	1.04 ± 0.32^{aA}
	BR		1.08 ± 0.05^{bC}	0.86 ± 0.04^{aB}	1.05 ± 0.05^{bA}
	ST		0.85 ± 0.03^{aA}	0.80 ± 0.01^{aA}	1.01 ± 0.05^{bA}

FA: Formic Acid; LP: *Lb. plantarum;* AC: *Pd. acidilactici*; GL: *Ent. gallinarum;* BR: *Lb. brevis* ST: *Streptococcus* spp
The values are expressed as mean ± standard deviation, n=3
Values in a same line followed by different letters (a-d) indicate significant differences of the parameter with respect to the storage time (P<0.05).
Values in a same column followed by different letters (A-D) indicate significant differences of the parameter with respect to the kind of silage (P<0.05).

were observed, it was recorded that these values were below the limit value (5 mg MDA eq./kg) according to Schormüller (1969). On the other hand, the TBARs value of FA group which was produced with formic acid was significantly higher than the fermented silages. It is suggested that lipid oxidation generally increases with the declining of pH because low pH promotes lipid oxidation. Jacobsen, Timm, and Meyer, (2001) reported that when pH value is decreased below 6.0, the iron bridges are broken, and iron ions become more active at lower pH levels for to promote the oxidation. Similar observations were detected at the second and third weeks in this study. Generally, the fluctuations defined in TBARs values of all groups might be emerged by the interactions of MDA with other components of muscle such as proteins, nucleic acids, nucleotides and other aldehydes. BHT which has the preventive effect concerning lipid peroxidation are commonly used as synthetic antioxidant. It can be also stated that the addition of BHT (250 mg/kg) was quite effective for the stabilization of lipids in fish silages in this study.

Microbiological Assessment of Fish Silages

E. coli, Salmonella spp., *Staph. aureus* and *Listeria* spp. were not detected in any analysed silage samples. Yeast and mold were also not found in tested samples. This could be due to the use of potassium sorbate in silage.

Initial TVC of seafood processing waste was 4.3 log cfu/g (Figure 1A). It was reported that fresh sardine processing waste contained 4.5×10^5 cfu/g total viable count (Zahar, Benkerroum, Guerouali, Laraki, & El Yakoubi, 2002). Application of formic acid resulted in a significant inhibition effect on bacterial growth in the first week of fermentation. At the end of the storage period, acid silage (FA) had 3.6 log cfu/g bacterial load. Significant reduction in bacterial number was also observed for fish visceral waste until the 4th week of storage (Bhaskar & Mahendrarkar, 2008). Santana-Delgado, Avila, and Sotelo, (2008) also observed a considerable reduction in aerobic bacterial growth in mackerel silage. A highly significant reduction in the total bacterial load in acid silage prepared from the dressing waste of fresh water fish with (Formic acid+Hydrochloric acid+Butyl Hydroxyl Toluene (FHB)) and without (Formic acid+Hydrochloric acid (FH)) the addition of synthetic antioxidant Butyl Hydroxyl Toluene (BHT) was reported on the 2nd day (Tanuja *et al*., 2014). Bello, Cardillo, and Martínez, (1993) also reported that fermented fish silage mixed with molasses, fruits (pineapple and papaya), sorbate and a starter of *Lb. plantarum* ATCC8014 showed only a few aerobic mesophilic organisms due to low pH values and a development of lactic acid bacteria. In the current study, among fermented silage the highest bacterial count was observed for ST (4.17 log cfu/g), whilst BR and GL had the lowest bacterial growth (3.8 and 3.9 log cfu/g, respectively) at the end of storage period.

Total anaerobic bacterial count was given in Figure 1B. Seafood processing waste initially had anaerobic bacterial count of 3.73 log cfu/g. Increases in anaerobic bacterial counts of all groups tested were observed throughout the storage period, especially at the end of the second week. Although total anaerobic bacterial count in acid silage reached to 5.0 log cfu/ml at the 3rd week, the bacterial load in fermented silages were above 8.6 log cfu/g. Among fermented silages, GL, ST and LP showed the highest anaerobic bacterial loads at the end of storage period.

Initial lactic acid bacteria count in seafood processing waste was 3.2 log cfu/g and remained below 6.2 log cfu/g in acid silage throughout the storage period (Figure 1C). However, at the end of storage the highest lactic acid bacteria counts were found to be 10.5 and 10.6 log cfu/g from ST and LP, respectively. Significant increases in lactic acid bacterial counts were also observed in sardine fillets inoculated with *Lactobacillus delbrueckii* subsp. *delbrueckii* during fermentation for two weeks

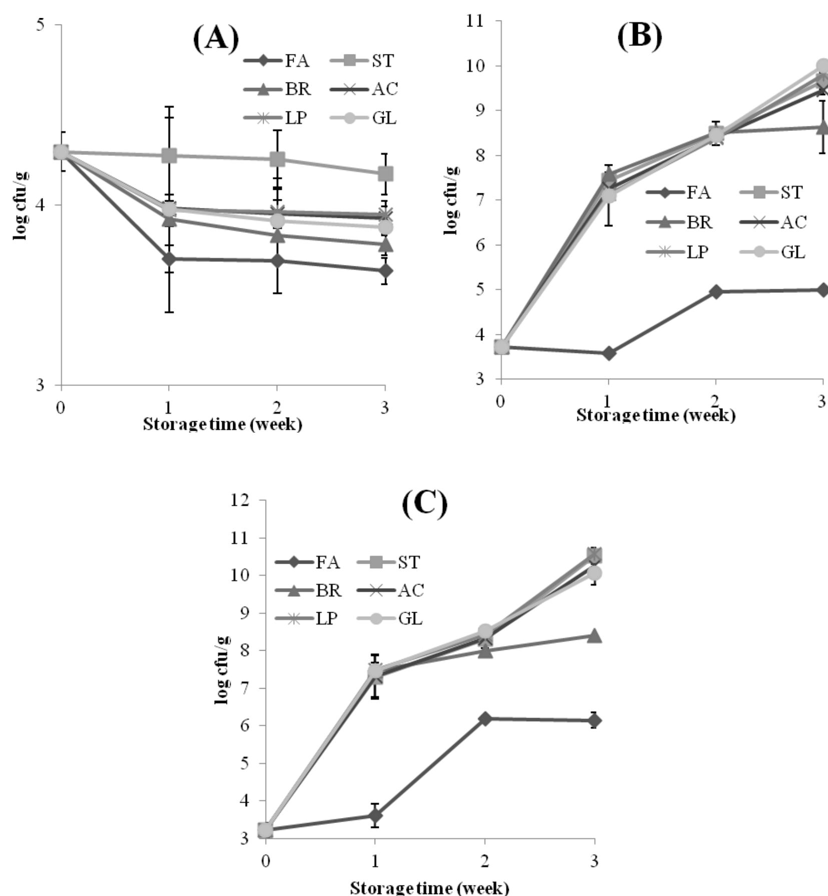

Figure 1. Microbiological changes in silage, A: total aerobic bacterial count, B: total anaerobic bacterial count, C: total lactic acid bacterial count.

(Ndaw, Faid, Bouseta, & Zinedine, 2008). The initial LAB count of 2.82 log cfu/g in discard fish reached their maximum values of 7.59 and 7.74 log cfu/g for fermented fish silage inoculated by *Lb. plantarum* and *Strep. thermophilus* at day 14, respectively (Özyurt *et al.*, 2016).

The silage fermentation totally inhibited the growth of coliform bacteria, although seafood processing waste initially had a 3.5 log cfu/g of coliform bacteria. Coliform bacteria was not detected in any silage samples. The absence of the indicator bacteria in fermented silage can be due to antimicrobial substances such as organic acids, hydrogen peroxide and bacteriocin as well as low pH (Earnshaw, 1992). Ndaw *et al.* (2008) found that coliform bacteria showed a net decrease during fermentation of sardine fillets inoculated with *Lactobacillus delbrueckii* subsp. *delbrueckii.*

Proximate and Amino Acid Compositions of Spray-Dried Fish Silages

Proximate compositions of acid and fermented spray-dried fish silages are shown in Table 3. Dry matter contents of spray-dried acid and fermented silages produced from sea bass wastes were found in the range of 89.60 to 91.32%. It was observed that there were some significant differences in dry matters among the groups ($P<0.05$). Generally, it is known that if the moisture contents of animal feeds are higher than 12%, it may cause a problem during prolonged storage. Moisture contents of dried fish silages in this study were determined below this value. Crude ash contents of spray-dried fish silages were found in the range of 8.73 and 9.84%. No significant differences were recorded in crude ash contents of all the groups ($P>0.05$). The high ash content of all silage groups is attributable to the high ash content of the raw material used in the silage preparation.

According to the technology that was used, it was reported that the fish meal might contain oil less than 1 percent or more than 20 percent (Polat, 2011). It is known that the rapid deterioration of fish meal may also be a result of the effects of high content of fish oil in fish meal. Therefore, it is generally preferred for the production of fish meal containing fat at a lower rate. In this study, after the lipid content of fish silage were extracted, the crude lipid content of spray-dried fish silages were found in range of 3.2%-3.66%. There were no significant differences of crude lipid content observed in acid and fermented

Table 3. Proximate (%) and Amino Acid Compositions (mg/100g sample) of Acid and Fermented Fish Silage Powders

Proximate Composition	FA	LP	AC	GL	BR	ST
Dry matter	90.51±0.78[bc]	90.18±0.27[ab]	91.32±0.31[d]	91.26±0.26[d]	90.82±0.82[cd]	89.60±0.60[a]
Crude ash	9.28±0.59[a]	9.06±0.78[a]	8.82±0.50[a]	8.91±0.44[a]	9.84±0.22[a]	8.73±0.43[a]
Crude Protein	30.88±0.06[a]	32.63±1.09[b]	32.70±0.56[b]	32.85±0.12[b]	32.94±0.06[b]	30.65±0.07[a]
Crude Lipid	3.66±0.01[a]	3.47±0.17[a]	3.32±0.29[a]	3.20±0.15[a]	3.60±0.07[a]	3.41±0.47[a]
Carbohydrate	46.69	45.02	46.48	46.30	44.44	46.81
Amino Acids						
Alanine	2122±28.28[a]	2784±43.13[e]	2457±5.66[b]	2589±13.44[c]	2417±22.63[b]	2650±0.00[d]
Glycine	2471±33.24[e]	2039±19.79[d]	1835±9.19[b]	2068±18.38[d]	1503±16.26[a]	1945±5.66[c]
Valine	1130±35.36[c]	1153±51.62[c]	1047±59.39[c]	932±74.95[b]	825±0.00[a]	936±4.24[b]
Leucine	2004±23.33[d]	1520±24.04[c]	1498±15.56[c]	1073±9.19[a]	1044±11.31[a]	1246±0.71[b]
Isoleucine	1361±25.46[e]	1299±4.95[d]	1212±0.71[c]	1082±0.71[b]	1020±0.71[a]	1211±8.49[c]
Threonine	844±10.61[c]	274±6.36[b]	252±3.54[b]	214±2.12[a]	193±18.38[a]	943±12.73[d]
Serine	1006±14.14[d]	295±10.61[c]	289±5.66[c]	300±9.89[c]	202±9.89[a]	235±9.19[b]
Proline	1591±4.95[e]	1327±8.49[cd]	1228±10.61[b]	1321±9.19[c]	1013±7.78[a]	1344±2.83[d]
Arginine	1058±2.83[de]	1071±26.87[e]	995±20.51[c]	1025±7.78[cd]	868±5.66[a]	914±14.14[b]
Aspartic acid	933±24.04[c]	714±15.56[a]	802±14.85[b]	986±8.49[d]	1009±16.97[d]	1442±19.09[e]
Methionine	425±13.44[e]	403±2.12[d]	336±0.71[c]	275±0.71[b]	258±2.12[a]	338±1.41[c]
Glutamic acid	1981±9.19[b]	1744±27.58[a]	1947±2.12[b]	2427±21.92[d]	2145±14.14[c]	2662±6.36[e]
Phenylalanine	1060±53.74[d]	629±45.25[c]	588±10.61[bc]	457±6.36[a]	430±7.78[a]	543±21.20[b]
Lysine	2546±103.95[d]	2411±101.12[cd]	2370±103.24[cd]	2243±79.90[bc]	2158±94.04[b]	1667±44.55[a]
Histidine	567±4.95[c]	415±4.95[b]	408±1.41[b]	415±1.41[b]	330±2.83[a]	337±0.71[a]
Tyrosine	772±41.72[e]	513±11.31[d]	416±10.61[b]	465±6.36[c]	490±0.71[cd]	269±4.95[a]

FA: Formic Acid; LP: *Lb. plantarum;* AC: *Pd. acidilactici*; GL: *Ent. gallinarum;* BR: *Lb. brevis* ST: *Streptococcus* spp
The values are expressed as mean ± standard deviation, n=3
Values in a same column followed by different letters (a-e) indicate significant differences of the parameter with respect to the kind of silage ($P<0.05$).

fish silage powders ($P>0.05$).

Crude protein content of spray-dried acid and fermented fish silages were determined in the range of 30.65%-32.94%. In generally, fish meals contain about 55-70% protein. It was reported that anchovy meal must contain at least 65% protein according to Turkish Standards Institute (TSE2033, Polat 2011). In this study, the spray-dried fish silages were produced with maltodextrin (1:1 w/w, on dry basis) for efficient drying. After that, the powdered fish silages had a mild fish aroma and didn't stick to the drying chamber of the spray dryer. Similar process for fish products were applied because of these advantages (Abdul-Hamid, Bakar, & Bee, 2002). Abdul Hamid *et al*. (2002) reported a similar result with our study that the protein ratio in tilapia protein hydrolysate dried with maltodextrin was 37.7%. Researchers were also reported that lipid and crude ash of spray-dried fish protein hydrolysate was 2.56% and 8.56%, respectively. While total crude protein, lipid and crude ash were calculated as 48.82%, carbohydrate ratio mainly due to maltodextrin was 49.6%. In this study, calculated carbohydrate ratios were in range of 45.02%-46.81%. Similarly, it was observed that in this study, total crude protein, lipid and crude ash were in the range of 42.79-45.16% and showed an equivalent ratio with carbohydrate percentages.

Fish is known to contain excellent amino acid compositions and is a unique source for essential amino acids such as lysine and methionine. Amino acid compositions of spray-dried acid and fermented fish silages are given in Table 3. Fish and crustaceans would be characterised by higher contents of leucine, lysine and alanine. Økland, Stoknes, Remme, Kjerstad, and Synnes, (2005) also reported that the dominant amino acid in fish muscle was lysine which its presence is limited in natural food, but relatively high amounts of aspartic acid, glutamic acid, arginine and leucine were also observed. In this study, glutamic acid, aspartic acid, leucine, alanine, glycine and lysine were the dominant amino acids (874-1055 mg/100 g sample) in raw fish waste (not seen in Table 3). Amino acid compositions did not show a homogenous trend after silage production. Similar results were observed by Morales-Ulloa and Qetterer (1997). It was observed that leucine, isoleucine, methionine, phenylalanine, glycine, serine, proline, histidine and tyrosine had significantly higher concentrations in FA group produced with formic acid than the other groups ($P<0.05$). In fermented fish silage powders, LP group produced with *Lactobacillus plantarum* had the highest isoleucine, methionine, tyrosine, arginine, glycine and alanine content. The essential/nonessential amino acid (E/NE) ratio of FA, LP, AC, GL, BR and ST group were found as 0.74, 0.71, 0.70, 0.54, 0.59 and 0.60 respectively. The low levels seen in the fermented fish silage powders in generally are thought to be caused by enzymatic activity of microorganisms and chemical reactions between amino groups and aldehyde groups during ensiling. E/NE ratio was found as 0.75-0.77 for sea bass muscle by Özyurt and

Polat (2006), 0.69 for sardine by Iwasaki and Harada (1985). If it is considered that the silage's raw material was the fish wastes and MD was added at 1:1 ratio (w/w, on dry basis) during drying, it can be concluded that the spray-dried fish silages have high quality and well balanced protein sources. In respect to E/NE ratio, the best groups among the spray-dried fish silages were FA, LP and AC groups prepared with formic acid, *Lactobacillus plantarum* and *Pediococcus acidilactici*, respectively.

Antioxidant Properties of Spray-Dried Fish Silages

Hydrolysed proteins produced from many animal and plant sources have been found to be in possession of antioxidant activity (Wu *et al*., 2003; Jamdar, Rajalakshmi, & Sharma, 2012). Fish silages are also protein hydrolysates with immune stimulating properties. Therefore, antioxidant properties of spray-dried fish silages were assessed with the basis of measuring total antioxidant activity (TAO) and scavenging of DPPH. TAO of spray-dried fish silage was compared with ascorbic acid (AA). According to this assay, TAO of spray-dried fish silages were 2.86, 2.26, 2.71, 2.44, 2.58 and 1.92 mg AA/g for FA, LP, AC, GL, BR and ST groups, respectively. The highest antioxidant activity was detected in FA group (P<0.05). Similarly, Umayaparvathi *et al*. (2014) recorded that the maximum TAO of bioactive peptides isolated from oyster hydrolysates (*Saccostrea cucullata*) was 2.1 mg AA/g.

DPPH which is a free radical has been used to evaluate the reducing substances for the comparison of the antioxidant capacity of proteins (Jamdar *et al*. 2012). DPPH shows maximum absorbance at 517 nm and reduction in absorbance is evaluated as a measure for radical-scavenging activity. The DPPH radical-scavenging ability of spray-dried fish silages were found as 7.55%, 8.42%, 20.26%, 16.95%, 7.89% and 7.87% for FA, LP, AC, GL, BR and ST groups, respectively. The highest DPPH inhibition rates were detected at AC and GL groups (P<0.05), but there were no significant differences observed in other groups (P>0.05). Sheriff, Sundaram, Ramamoorthy, & Ponnusamy, (2014) reported that the radical scavenging ability of pepsin and papain derived protein hydrolysates of Indian mackerel (*Rastrelliger kanagurta*) was found to be a maximum of 46% and 36%, respectively. Samar, El-Kalyoubi, Khalaf, & El-Razik (2013) recorded that the radical scavenging activity of chitosan samples extracted from shrimp wastes using microwave technique were in rage of 16.14%-32.76% (approximately 23.68%) at same concentrations. Ghorbel-Bellaaj, Younes, Maâlej, Hajji, and Nasri, (2012) also emphasised that peptides present in the fermented seafood wastes are good electron donors and they can terminate the radical chain reactions by converting free radicals into more stable products. According to the data obtained in this study, it can be concluded that acid and fermented silage powders are valuable feed sources due to their antioxidant properties.

Antimicrobial Activity of Spray-Dried Fish Silages

Antibacterial activity of the spray-dried fish silages were analysed by the test strains using paper disk diffusion test and the broth microdilution method. An inhibition zone against the test strains was not observed in all spray-dried fish silages. As positive control samples, inhibition zones of tetracycline and neomycine were 2.0 and 2.1 mm for *Staph. aureus*, 2.6 and 2.0 mm for *C. jejuni*, 1.8 and 1.5 mm for S. *paratyphi* A, 2.2 and 1.8 mm for *K. pneumoniae*, 2.4 and 1.9 mm for *Y. enterocolitica*, 2.0 and 1.7 mm for *E.coli,* 2.5 and 2.0 mm for *Ent. faecalis,* and 2.1 and 1.9 mm for *P. aeroginosa,* respectively (Figure 2). Similarly according to broth microdilution method, all inoculated tubes showed turbidity, indicating a failure of spray-dried fish silage for the inhibition of bacterial growth even at 50 mg/mL concentration. The production levels and the proportions of antimicrobial compounds produced by lactic acid bacteria depend on the strain, medium

Figure 2. Antimicrobial activity of spray-dried fish silages, A: neomycine, B: tetracycline, C: spray-dried silages.

compounds and physical parameters (Tannock, 2004; Kazemipoor, Radzi, Begum, & Yaze, 2012).

In-vitro Gas Production of Spray-Dried Fish Silages

The amounts of gas production depending on time which varied depending on the nutrient contents are shown in Figure 3. It was observed that the LP group (50.97±2.30 ml) had the lowest gas production value after 24 hours (P<0.05). There were no significant differences of the gas production values among the other groups after 24 hours (P>0.05). The LP group still had the lowest gas production value after 96 hours (58.58±1.32) (P<0.05). The parameters of gas production and OMD, ME and NE_L values of spray-dried silages are given in Table 4. The OMD, ME and NE_L values of spray-dried fish silages were found in the rage of 84.31-87.76%, 9.38-10.12 MJ/kg DM and 7.80-8.12 MJ/kg DM, respectively. The lowest OMD, ME and NE_L values observed in LP group (P<0.05) and the other groups did not show any significant differences.

Kılıc, Garipoglu, Boga, and Yurtseven (2008) reported in a study about fish meal that they had obtained 3.67 MJ/kg for ME and 59.50% for OMD. In this study, even if some differences observed between silage groups, it was determined that all groups generally had better digestibility rate compared to fish meal reported by Kılıc *et al*. (2008). It was believed that this situation was originated by the added maltodextrin which was the source of the carbohydrate and was used for as a drying agent to help creating the spray-dried silages. In accordance with that the raising gas production of the feed is thought to be because of the increase in carbohydrate rates. It is known that protein fraction produces less gas, fat fraction produces less gas and ash doesn't ferment (Robinson & Getachew, 2002). Therefore, it was determined that spray-dried fish silages prepared with this method were more suitable for feeding farm animals compared to fish meal.

Spray-dried silages were seen as an important raw material for the usage of animal feed because of their high crude protein contents. It was necessary for crude protein ratio to be at least 10% in feed for the growth of the animal and for the optimization of rumenic microbial activities (Norton, 2003). For this reason, if the crude protein level is below 10% in feed, microbial activity will decrease and therefore it will lead to a decrease at gas production (Kılıc & Boga, 2009). The feed that was used in this study was considered an important source for animal nutrition because of its rich crude protein content. The difference among the spray-dried silages used in this study would be because of the varied enzymatic activities of the lactic acid bacteria that were used. Similar opinions were also pointed out about the low gas production in some other studies (Muck, 2008; Sucu, 2009).

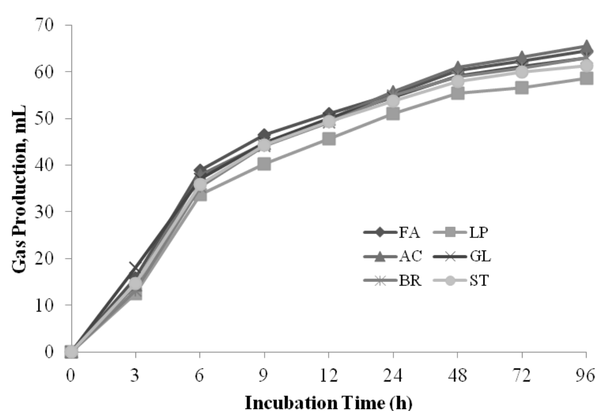

Figure 3. Gas production values of fish silages.

Table 4. In vitro gas production parameters and OMD, ME and NE_L of fish silages

Silage groups	a ml	b ml	c ml/saat	rsd	OMD %	ME MJ/kg DM	NE_L MJ/kg DM
FA	-6.51±5.36^{b}	77.21±5.48^{a}	0.19±0.02^{ab}	3.30	86.17±0.44^{ab}	10.02±0.09^{a}	8.05±0.04^{a}
LP	-4.86±2.33^{b}	70.53±3.74^{bc}	0.17±0.01^{ab}	2.69	84.31±1.75^{b}	9.38±0.36^{b}	7.80±0.17^{b}
AC	-3.84±3.02^{b}	75.59±2.02^{ab}	0.17±0.02^{b}	3.49	87.76±0.34^{a}	10.12±0.07^{a}	8.13±0.03^{a}
GL	-7.14±1.35^{a}	67.05±0.24^{c}	0.17±0.01^{b}	2.65	86.76±1.51^{a}	9.94±0.31^{a}	8.05±0.15^{a}
BR	-7.95±3.27^{b}	77.61±2.15^{a}	0.18±0.01^{ab}	2.66	85.99±0.59^{ab}	9.98±0.12^{a}	8.03±0.06^{a}
ST	-18.82±2.94^{b}	76.36±1.86^{a}	0.19±0.01^{a}	2.49	86.50±0.91^{a}	9.83±0.19^{a}	8.05±0.09^{a}
Sig	.011	.006	.094		.038	.020	.038

FA: Formic Acid; LP: *Lb. plantarum;* AC: *Pd. acidilactici*; GL: *Ent. gallinarum;* BR: *Lb. brevis* ST: *Streptococcus* spp
The values are expressed as mean ± standard deviation, n=3
Values in a same column followed by different letters (a-c) indicate significant differences of the parameter with respect to the kind of silage (P<0.05).

It was reported that the observed differences of in vitro gas production of feed were caused by the proximate composition, whether the grains contained husks or not and the starch content and also in vitro gas production was observed to be lower when feed stuff was processed by a high degree of heat treatment and when the plants matured. (Menke & Steingass, 1988; Getachew, Robinson, DePeters, & Taylor, 2004). As a result, when the data about in vitro digestibility of acid and fermented spray-dried fish silages generally evaluated, it can be said that crude protein content and digestibility of spray-dried silages were high and they were valuable sources as feeding materials for animal nutrition.

Conclusion

It was concluded that five LAB strains (*Lb. plantarum, Pd. acidilactici, Ent. gallinarum, Lb. brevis* and *Streptococcus* spp.) selected as starter cultures for the production of fish silage fermentation were found to be efficient strains. It was a first for *Ent. gallinarum* to be used for this purposes. Feed is the major cost for livestock production, particularly protein is the most expensive nutritional component for feed formulations. As a conclusion, regarding to data obtained from proximate compositions, amino acid compositions, antioxidant activity and in-vitro digestibility analysis, acid and fermented fish silage powders could be used as a valuable and economical food sources for animal production.

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References

Abdul-Hamid, A., Bakar, J., & Bee, G.H. (2002). Nutritional Quality of Spray Dried Protein Hydrolysate from Black Tilapia (*Oreochromis mossambicus*). *Food Chemistry*, 78(1), 69-74. http://dx.doi.org/10.1016/S0308-8146(01)00380-6.

Akman, N. (2013). Türkiye'de Hayvansal Üretim, Türkiye'de Tarımın Ekonomi Politiği 1923–2013. N. Oral, (Ed.), Ankara, Türkiye, TMMOB Ziraat Mühendisleri Odası Bursa Şubesi ve Nota Bene Yayınları 213–247 pp.

AOAC. (1999). Official Method 981.10 Crude Protein in Meat, In: P. Cunniff. (Eds), Meat and Meat Products, Official Methods of Analysis of the AOAC International, vol. II. 16th ed., Chapter 39, Gaithersburg, MD: 1–15.

AOAC. (2002). Official Methods of Analysis 16th Edn. Association of Official Analytical Chemists. Washington, DC. USA.

Bello, R., Cardillo, E., & Martínez, R. (1993). Microbial Silage Production from Eviscerated Fish. *Archivos latinoamericanos de nutricion*, 43(3), 221-227.

Bhaskar, N., & Mahendrakar, N.S. (2007). Chemical and Microbiological Changes in Acid Ensiled Visceral Waste of Indian Major Carp *Catla catla* (Hamilton) with Emphasis on Proteases. *Indian Journal of Fisheries*, 54(2), 217-225.

Bhaskar, N., & Mahendrakar, N.S. (2008). Protein Hydrolysate from Visceral Waste Proteins of Catla (*Catla catla*): Optimization of Hydrolysis Conditions for a Commercial Neutral Protease. *Bioresource Technology*, 99(10), 4105-4111. http://dx.doi.org/10.1016/j.biortech.2007.09.006

Bligh, E.G., & Dyer, W.J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.

Clinical and Laboratory Standards Institute (2008). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, CLSI, Wayne, Pa, USA.

Close, W., & Menke, K.H. (1986). Selected Topics in Animal Nutrition. *Deutsche Stiftung Für Internationale Entwicklung*, Dok 1350 C/a, Germany, 170 pp.

Crexi, V.T., Souza-Soares, L.A., & Pinto, L.A.A. (2009). Carp (*Cyprinus carpio*) Oils Obtained by Fishmeal and Ensilage Processes: Characteristics and Lipid Profiles. *International journal of food science & technology*, 44, 1642–1648. http://dx.doi: 10.1111/j.1365-2621.2009.01982.x

Dimova, N. (2003). RP-HPLC Analysis of Aminoacids with UV-Detection. *Comptes Rendus de l'Academie Bulgare des Sciences*, 56(12), 75.

Earnshaw, R.G. (1992). The Antimicrobial Action of Lactic Acid Bacteria: Natural Food Preservation Systems. *The Lactic Acid Bacteria Volume 1*, Springer US, 211-232.

EC. (2002). The use of fish by-products in aquaculture. Retrieved from http://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1461238051153&uri=CELEX:32009R1069.

El-Ajnaf, S.M. (2009). Development of Fermented Fish Silage Derived from Pelagic Sardine and Apple Pomace by-product for Inclusion in Diets for the European Sea Bass (*Dicentrarchus labrax*). *University of Plymouth, Faculty of Science and Technology*, PhD Thesis, 201 pp.

Getachew, G., Robinson, P.H., DePeters, E.J., & Taylor, S.J. (2004). Relationships Between Chemical Composition, Dry Matter Degradation and in Vitro Gas Production Of Several Ruminant Feeds. *Animal Feed Science and Technology*, 111(1), 57-71. http://dx.doi.org/10.1016/S0377-8401(03)00217-7

Ghorbel-Bellaaj, O., Younes, I., Maâlej, H., Hajji, S., & Nasri, M. (2012). Chitin extraction from shrimp shell waste using Bacillus Bacteria. *International journal of biological macromolecules*, 51(5), 1196-1201. http://dx.doi.org/10.1016/j.ijbiomac.2012.08.034

Goosen, N.J., Wet, L. F., & Görgens, J.F. (2016). Rainbow trout silage as immune stimulant and feed ingredient in diets for Mozambique Tilapia (*Oreochromis mossambicus*). *Aquaculture Research*, 47(1), 329-340. doi: 10.1111/are.12497

Iwasaki, M., & Harada, R. (1985). Proximate and amino acid composition of the roe and muscle of selected marine species. *Journal of Food Science*, 50(6), 1585-1587. doi: 10.1111/j.1365-2621.1985.tb10539.x

Jacobsen, C., Timm, M., & Meyer, A.S. (2001). Oxidation in fish oil enriched mayonnaise: Ascorbic acid and low pH increase oxidative deterioration. *Journal of*

Agricultural and Food Chemistry, 49(8), 3947-3956. doi: 10.1021/jf001253e

Jamdar, S.N., Rajalakshmi, V., & Sharma, A. (2012). Antioxidant and Ace Inhibitory Properties of Poultry Viscera Protein Hydrolysates and Its Peptide Fractions. *Journal of Food Biochemistry*, *36*(4), 494-501. doi: 10.1111/j.1745-4514.2011.00562.x

Jangaard, P.M. (1987). Le poisson ensile: historique et developpements recents. In Anon Rapport (Eds), *Attelier sur l'ensilage du poisson* (pp. 8-37). Pointe-de-1'Eglise, NouvelleEcosse, 16-17 Juin, 1987. Min. Peches Oceans, Canada, Series Education Gen.

Kazemipoor, M., Radzi, C.W.J.W.M., Begum, K., & Yaze, I. (2012). Screening of Antibacterial Activity of Lactic Acid Bacteria Isolated from Fermented Vegetables Against Food Borne Pathogens. *Archives Des Sciences*, 65(6), 453-466. arXiv:1206.6366v1

Kılıc, U., & Boga, M. (2009). Protein İçeriği Farklı Ayciceği Tohumu Küspelerinin İn Vitro Gaz Üretim Tekniği ve Enzim Tekniği Sonuçları Üzerine Etkilerinin Belirlenmesi. *Tubav Bilim Dergisi*, 2 (2), 231-238.

Kılıc, U., Garipoglu, A.V., Boga, M., & Yurtseven, S. (2008). Bazı Yem Hammaddelerinin in Vitro Gaz Üretimi, Rumen Parçalanabilirlikleri ve Enerji İceriklerinin Belirlenmesi. *Ondokuz Mayıs Üniversitesi, 4. Ulusal Zootekni Öğrenci Kongresi*, 248-253.

Menke, K.H., & Steingass, H. (1988). Estimation of the Energetic Feed Value Obtained from Chemical Analysis and in Vitro Gas Production Using Rumen Fluid. *Animal Research and Development*, 28, 7-55.

Morales-Ulloa, D.F., & Oetterer, M. (1997). Composição em Aminoácidos de Silagens Químicas, Biológicas e Enzimáticas Preparadas com Resíduos de Sardinha. *Sociedade Brasileira de Ciência e Tecnologia de Alimentos*, 17(3), 252-258.

Muck, R.E. (2008). Improving Alfalfa Silage Quality with Inoculants and Silo Management. *Cornell Nutrition Conference*, (21-23). Syracuse, NY,137-146.

Murray, P.R., Baron, E.J., & Pfaller, M.A. (1995). Manual of Chinical Microbiology, 6th ed. ASM, Washington DC.

Murthy, P.S., Rai, A.K., & Bhaskar, N. (2014). Fermentative Recovery of Lipids and Proteins from Freshwater Fish Head Waste with Reference to Antimicrobial and Antioxidant Properties of Protein Hydrolysate. *Journal of Food Science and Technology*, 51(9), 1884-1892. doi: 10.1007/s13197-012-0730-z

Ndaw, A.D., Faid, M., Bouseta, A., & Zinedine, A. (2008). Effect of controlled lactic acid bacteria fermentation on the microbiological and chemical quality of Moroccan sardines (*Sardina pilchardus*). *International Journal of Agriculture & Biology*, 10, 21-27. http://dx.doi.org/10.1556/AMicr.55.2008.3.2

Nørgaard, J.V., Petersen, J.K., Tørring, D.B., Jørgensen, H., & Lærke, H.N. (2015). Chemical Composition and Standardized Ileal Digestibility of Protein and Amino Acids from Blue Mussel, Starfish and Fish Silage in Pigs. *Animal Feed Science and Technology*, 205, 90-97. http://dx.doi.org/10.1016/j.anifeedsci.2015.04.005

Norton, B.W. (2003). The nutritive value of tree legume. Retrieved from http://www.fao.org/ag/AGP/AGPC/doc/Pub/licat/Gutt-shel/x5556e0j.htm

Økland, H.M., Stoknes, I.S., Remme, J.F., Kjerstad, M., & Synnes, M. (2005). Proximate Composition, Fatty Acid and Lipid Class Composition of the Muscle from Deep-sea Teleosts and Elasmobranches. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *140* (3), 437-443. http://dx.doi.org/10.1016/j.cbpc.2004.11.008

Orskov, E.R., & McDonald, I. (1979). The Estimation of Protein Degradability in the Rumen from Incubation Measurements Weighted According to Rate of Passage. *The Journal of Agricultural Science*, 92(02), 499-503. https://doi.org/10.1017/S0021859600063048

Ozyurt, G. (2016). Biotransformation of Fisheries By-products Fermented with Natural Lactic Acid Bacteria, the Quality of Fermentation Products and Their Use in Animal Feeding. Final Report of TUBITAK) Project (213O166), 128 pp.

Ozyurt, G., & Polat, A. (2006). Amino Acid and Fatty Acid Composition of Wild Sea Bass (*Dicentrarchus labrax*): A Seasonal Differentiation. *European Food Research and Technology*, 222 (3-4), 316-320. doi: 10.1007/s00217-005-0040-z

Ozyurt, G., Gökdoğan, S., Şimşek, A., Yuvka, I., Ergüven, M., & Kuley, E. (2016). Fatty Acid Composition and Biogenic Amines in Acidified and Fermented Fish Silage: A Comparison Study. *Archives of Animal Nutrition*, 70 (1), 72-86. http://dx.doi.org/10.1080/1745039X.2015.1117696

Perez, R. (1995). Fish Silage for Feeding Livestock. *World Animal Review*, 82, 34-42.

Polat, A. (2011). Yemler Bilgisi ve Karma Yem Teknolojisi. Ç.Ü. Su Ürünleri Fakültesi Ders Kitabı, Adana Nobel Kitabevi, 139 pp.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric Quantitation of Antioxidant Capacity Through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Analytical Biochemistry*, 269 (2), 337-341. doi:10.1006/abio.1999.4019

Raa, J., & Gilberg, A. (1982). Fish Silage: A review. *Crc Critical Reviews In Food Science and Nutrition,* 16, 383-420.

Rai, A.K., Bhaskar, N., Halami, P.M., Indirani, K., Suresh, P.V., & Mahendrakar, N.S. (2009). Characterization and Application of a Native Lactic Acid Bacterium Isolated from Tannery Fleshings for Fermentative Bioconversion of Tannery Fleshings. *Applied Microbiology and Biotechnology,* 83(4), 757-766. doi: 10.1007/s00253-009-1970-3.

Robinson, P.H., & Getachew, G. (2002). A practical gas production technique to determine the nutritive value of forages. *Proceedings of the National Forage Testing Association Annual Meeting*, 8 pp.

Sachindra, N.M., & Bhaskar, N. (2008). In Vitro Antioxidant Activity of Liquor from Fermented Shrimp Biowaste. *Bioresource Technology*, 99, 9013-9016. http://dx.doi.org/10.1016/j.biortech.2008.04.036

Salas, G., Gutiérrez, E., Juárez, A., Flores, J.P., & Perea, M. (2011). Use of the Devil Fish in Animal Feed as an Alternative to Productive Diversification and Mitigation of Environmental Damage in the South and West of México. *Journal of Agricultural Science and Technology A*, 1232.

Samar, M.M., El-Kalyoubi, M.H., Khalaf, M.M., & El-Razik, M.A. (2013). Physicochemical, Functional, Antioxidant and Antibacterial Properties of Chitosan Extracted from Shrimp Wastes by Microwave Technique. *Annals of Agricultural Sciences*, 58(1),

33-41. http://dx.doi.org/10.1016/j.aoas.2013.01.006

Santana-Delgado, H., Avila, E., & Sotelo, A. (2008). Preparation of Silage from Spanish Mackerel (*Scomberomorus maculatus*) and Its Evaluation in Broiler Diets. *Animal Feed Science and Technology*, 141 (1), 129-140. http://dx.doi.org/10.1016/j.anifeedsci.2007.05.023

Schormüller, J. (1969). Handbuch der Lebensmittelchemie (Band III/2). Triesrische Lebensmittel Eier, Fleisch, Fisch, Buttermich, pp.1561–1578, 1584. Springer Verlag, Berlin/Heidelberg, Germany/New York, NY.

Sheriff, S.A., Sundaram, B., Ramamoorthy, B., & Ponnusamy, P. (2014). Synthesis and In Vitro Antioxidant Functions of Protein Hydrolysate from Backbones of Rastrelliger Kanagurta by Proteolytic Enzymes. *Saudi Journal of Biological Sciences*, 21(1), 19-26. doi:10.1016/j.sjbs.2013.04.009

Shirai, K., Guerrero, I., Huerta, S., Saucedo, G., Castillo, A., Gonzalez, R.O., & Hall, G. (2001). Effect of Initial Glucose Concentration and Inoculation Level of Lactic Acid Bacteria in Shrimp Waste Ensilation. *Enzyme and Microbial Technology,* 28, 446-452. doi:10.1016/S0141-0229(00)00338-0

Sucu, E. (2009). Laktik Asit Bakteri İnokulantlarinin Misir Silajinin Fermantasyon ve Aerobik Stabilite Özellikleri ile Rumen Ekolojisi Üzerine Etkileri. PhD Thesis, *Uludağ University*, Bursa, 145 pp.

Tannock, G.W. (2004). A special fondness for lactobacilli. *Applied and Environmental Microbiology*, 70(6), 3189-3194.

Tanuja, S., Mohanty, P.K., Kumar, A., Moharana, A., & Nayak, S.K. (2014). Shelf life study of acid added silage produced from fresh water fish dressing waste with and without the addition of antioxidants. *International Journal of Agriculture and Food Science Technology*, 5, 91-98.

Tarladgis, B., Watts, B.M., & Yonathan, M. (1960). Distillation method for determination of malonaldehyde in rancid food. *Journal of the American Oil Chemists' Society,* 37, 44-48. doi:10.1007/BF02630824

Tejeda-Arroyo, E., Cipriano-Salazar, M., Camacho-Díaz, L.M., Salem, A.Z.M., Kholif, A.E., Elghandour, M.M.M.Y., & Cruz-Lagunas, B. (2015). Diet Inclusion of Devil Fish (*Plecostomus spp.*) Silage and Its Impacts on Ruminal Fermentation and Growth Performance of Growing Lambs in Hot Regions of Mexico. *Tropical animal Health and Production*, 47(5), 861-866, 47(5), 861-866. doi:10.1007/s11250-015-0800-0

Van Hylckama Vlieg, J.E.T., & Hugenholtz, J. (2007). Mining Natural Diversity of Lactic Acid Bacteria for Flavour and Health Benefits. *International Dairy Journal*, 17, 1290-1297. doi:10.1016/j.idairyj.2007.02.010

Wu, H.C., Chen, H.M., & Shiau, C.Y. (2003). Free Amino Acids and Peptides as Related to Antioxidant Properties in Protein Hydrolysates of Mackerel (*Scomber austriasicus*). *Food Research International*, 36(9), 949-957. doi:10.1016/S0963-9969(03)00104-2

Yusuf, M.A., & Hamid, T.H. (2013). Lactic Acid Bacteria: Bacteriocin Producer: A Mini Review. *IOSR Journal of Pharmacy*, 3(4), 44-50.

Zahar, M., Benkerroum, N., Guerouali, A., Laraki, Y., & El Yakoubi, K. (2002). Effect of Temperature, Anaerobiosis, Stirring and Salt Addition on Natural Fermentation Silage of Sardine and Sardine Wastes in Sugarcane Molasses. *Bioresource Technology*, 82(2), 171-176. doi:10.1016/S0960-8524(01)00165-1