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A STUDY ON CHEMICAL AND MICROBIOLOGICAL COMPOSITION OF SILAGE MADE OF INDUSTRIAL FISHERIES PROCESSING WASTE

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Abstract

The growing demand in fish meal leads to increase in its price. This increase results in the search of raw materials that can be an alternative to fish meal. It is highly important to produce fish silage out of wastes of processing industry and use it in fish feed industry as an alternative stuff to some of fish meal. This study aims to analyse the chemical and microbiological properties of fish silage produced out of wastes of processing industry. This study puts forward that protein content of the silage is lower than that of fish meal. However, its amino acids profile revealed that fish silage have high quality protein content. Following its storage, the fish silage lipid content reached to 32.71% with a level of 25% PUFA and having 9% omega 3 fatty acid and 3.7% omega 6 fatty acid rates. It was seen that there is no microbiologically problem, like an increase in the number of bacteria or another negative change in microbiological quality parameters, to use the produced silage immediately after its maturity or storage. This study brings forward that the fish silage produced out of wastes of processing industry can be used as an alternative substance to fish meal if its protein deficit is met by protein concentrators.
Keywords: Fish meal, sustainability, fisheries economy, protein quality

SU ÜRÜNLERİ İŞLEME SANAYİ ATIKLARINDAN ÜRETİLEN SİLAJIN KİMYASAL VE MİKROBİYOLOJİK KOMPOZİSYONUNUN ARAŞTIRILMASI ÜZERİNE BİR ÇALIŞMA

Özet

Balık ununa olan talebin her geçen gün artması balık unu fiyatlarında artışa sebep olmaktadır. Bu artış, balık ununa alternatif olabilecek hammaddelerin aranmasına neden olmaktadır. İşleme sanayi atıklarından silaj yapılması ve yem sanayinde balık ununun bir kısmı yerine alternatif hammadde olarak kullanılması oldukça önemlidir. İşleme sanayi atıklarından üretilen silajın kimyasal ve mikrobiyolojik özelliklerinin incelenmesi bu çalışmanın amacıdır. Yapılan çalışmada üretilen silajın protein değeri balık ununa göre düşük bulunmuştur. Ancak aminoasit dağılımına bakıldığında yüksek kalitede protein içerdiği tespit edilmiştir. İşleme atığı silajının depolama sonucunda ham yağ değeri %32,71, PUFA değeri %25, omega 3 yağ asidi miktarı %9 ve omega 6 yağ asidi miktarı %3,7 olarak belirlenmiştir. Üretilen silajın olgunlaşmasından hemen sonra veya depolamadan sonra kullanılmasında bakteri sayısında artma veya mikrobiyolojik kalite parametrelerinde olumsuz bir değişim olmadığı için mikrobiyolojik açıdan bir problem olmadığı görülmüştür. Bu çalışmada işleme sanayi atıklarından üretilen silajın protein açığının protein zenginleştiricilerle giderilmesi durumunda balık ununa alternatif bir hammadde olabileceği ortaya konulmuştur.
Anahtar Kelimeler: Balık unu, sürdürülebilirlik, su ürünleri ekonomisi, protein kalitesi

1 Introduction

Developing new resources and using the readily available ones in the best way possible is now one of the most prioritized concerns in all areas. In Turkey, a significant portion of processed seafood, 168.073 tons exactly, is used to manufacture fish oil and fish meal [1]. However, the amount of fish meal manufactured does not completely meet the need by fish feed producers. Fish meal import has become a necessity in order to compensate for fish protein deficit. Meanwhile, fish meal price has been increasing due to the diminishing of fish stock and increasing demand for the product. Therefore, feed manufacturers cut down on the use of fish meal that added in fish feed, which in turn degrades feed quality. So far, many studies have been conducted to find out alternative raw material sources to replace fish meal.

In fish processing nearly 60% of the processed fish turns out as waste material. As a result of this processing activity, a significant amount of unused waste piles up. Re-processing this waste into fish silage and using the silage to replace fish meal in feed industry is bound to bring in substantial gains and enable the use of waste for a good purpose on low costs [2]. Not only the processing waste, but also heaps of spoiling seafood which could not have been sold in the market on time and lost their fresh qualities can be processed in this manner. Industrial processing waste was used to manufacture fish meal for feeding animals [3]. However, due to the fact that costs of manufacturing fish meal are high and the quality of fish meal obtained from fisheries processing waste do not meet the

expectations, the method of converting fisheries processing waste into fish meal has not been developed so far.

Fish silage is a biological product that is obtained by liquidizing seafood not offered for human consumption and also waste from seafood processing [4-5]. Fermentation bacteria and the acid-induced maturation method are used to make fish silage [4, 6].

The first fish silage production started in Finland in 1920. Up to now, characteristics of this silage have been a matter of research at different times [7, 8]. It was reported that the acid used in manufacture of silage for the purpose of reducing pH melts down bones and cartilages and also prevents growth of mould and bacteria [4, 8].

It was noted that over time, proteins are decomposed into soluble peptic products, free amino acids, ammonium and other metabolic products during prolonged storage of matured silage [8, 9].

It is also noted that some amino acids (tryptophan, phenylalanine, tyrosine and arginine) are catabolized [8, 10].

Additionally, it was recorded that the silage will feature thiamine deficiency due to the fact that the B1 vitamin is decomposed by the thiaminase enzyme during silage production, but thermal processing of silage raw material will be useful to counter such deficiency [8, 11-12]. Suggestions to prevent catabolism other than practicing thermal processing include pasteurization [8, 13], addition of formalin [14] or various enzyme inhibitors [8, 15] or further reducing of pH value [8, 9].

In some applications, fish silage is mixed with marine macro algae and processed soy flour to be used as a nutritional enrichment in feed. The general purpose of silage studies is to reduce the need for fish meal in fish feed as much as possible and develop a feed raw material by utilizing local sources of protein [16-18]. Feed raw materials of high quality are not only expensive but also difficult to procure. Other primary sources of protein than fish meal are soy flour, cottonseed flour, meat meal and feather meal. Marine macro algae or wild fresh water plants like hyacinth, whose habitats are very broad, can also be used as new feed raw material sources. These are used as protein sources particularly for tilapia and gray mullet in local regions [18]. Fish silage contains high nutritional value substances and displays a balanced essential amino acid structure. Therefore, it is very useful as a protein supplement in fish feeding [5, 19-22].

Since the nutrient content of waste is very high, monitoring microbiological quality of the waste became necessary. In some studies, it was reported that potassium sorbate and etoxiquin are added in the mixture in order to prevent formation of mold/fungi and oxidation [22]. The course of microbial activity that emerges as a result of the pH reduction induced by the influence of acid is a topic that should be looked into at silage storage stage.

Fish silage is an excellent source in terms of both protein and fat. Despite the fact that there are numerous studies available on fish and fish oils, studies on utilization of industrial processed seafood waste as silage are little in number. Silage production efforts important in terms of preventing fish oil rancidity, protecting valuable fatty acids, utilizing available raw material and converting it into a product of better quality [23].

In this research, the changes in chemical and microbiological characteristics of silage, which was manufactured to utilize industrial seafood processing waste, were observed over time during storage.

2 Material and Method

In this research, unprocessed pearl mullets and fisheries waste were used. These materials were minced into separate batches using a meat grinder. Then, in accordance with Turkish Food Regulations, 1.5% of weight formic acid (85% purity) and 1.5% of weight sulphuric acid (98% purity) were added to each batch (w/v). As decomposition was bound to emerge in spots inaccessible to acid, acid was dispersed in the minced product in a homogenous manner. The pH value of the minced batches was reduced below four and efforts were made to maintain at this pH level.

The production of silage was carried out above the room temperature, in order to prevent oxidation; BHT (butyl hydroxy-toluene) was added to the minced batches at dosages of 300 ppm as an antioxidant material [23]. The obtained mixtures were then put in separate lidded plastic storage containers with a volume of 60 L each. After lids of these containers, which are resistant to acidic wearing, were closed, then stored at room temperature. Samples were taken on five occasions for the purpose of chemical analyses at the beginning, on the 30th, 60th y, 90th and 150th days respectively. Before extracting samples, the products were mixed homogeneously. Representative samples (250 g) were drawn from each container and placed into protective bags. The protective bags were kept in a deep freezer (-18°C) until further analyses. For microbiological analyses, samples of 100 g were taken from the homogenously mixed products seven times in total, in the beginning, on the 7th, 15th, 30th, 60th, 90th and 120th days respectively. They were put in sterile stomacher bags and their culture studies were conducted in the Food Hygiene Laboratory of the Veterinary Faculty of Yüzüncü Yıl University. Samples taken for chemical analyses were sent to TUBITAK Marmara Research Center (MAM) for their crude protein, amino acid, crude fat, fatty acid composition and vitamin B1 (thiamine) analyses in compliance with sample dispatching conditions.

2.1 Amino acid analysis

The crude proteins of samples were determined by the Kjeldahl method, and were calculated using a nitrogen conversion factor of 6.25 [24]. The results were expressed as per cent of dry weight basis.

In order to determine the amino acid composition, between 10 mg and 15 mg of the dried samples according to contents of crude protein which should be contain 30% was hydrolysed at 110°C for 24 hours with 6.0 M hydrochloric acid. The hydrolysates of all samples was filtered through a 0.20 µm PTFE syringe filter, and then was evaporated all the hydrochloric acid in the hydrolysates. After evaporation, all hydrolysate samples were dissolved in citrate–sodium citrate buffer (0.1 M, pH 2.2) [25].

The level of amino acids were measured in samples using EZ:fast kits (EZ:fast GC/FID Protein Hydrolysate Amino Acid Kit) by gas chromatography according to Badawy et al. (2008). The procedure of amino acids analysis consists of a solid phase extraction step, followed by a derivatization procedure and a liquid/liquid extraction step [26]. Prepared and derivatized samples were then analysed by gas chromatography. Norvaline is used as an internal standard. The concentration of the internal standard (IS; Norvaline) in the sample prepared for GC analysis was used as 200 nmoles/mL. GC was used to determine amino acid composition. The 10 m x 0.25 mm capillary GC column was used. GC conditions were performed as injection: Split 1:15 at 250ºC, 2.0 µL; carrier gas: helium 1.0 mL/min;

oven program: 35ºC/min from 110ºC to 320ºC, hold at 320º for 1 min; Detector: FID at 320ºC. The instrument was calibrated with standard solution of multi amino acids (EZ:fast SD solutions).

Fatty acid analysis:

Crude fat analysis was performed using the Bligh and Dyer (1959) method in order to find out the chemical compositions of the silage samples [27]. Fatty acid analysis was performed on the basis of the IUPAC II.D.19 method [28-29].

Fatty acid content was analysed in a GC device together with the use of methyl esters (FAME), a flame ionization detector (FID) and a DB-23 capillary column (60 m, 0.25 mm i.d. and 0.25 µm). Temperatures of the injector and detector were 270 and 280 °C respectively. Column temperature remained at 190 °C for 35 minutes and was later raised to 220 °C with an increasing rate of 30 °C/min. It remained at the final temperature for 5 minutes. Helium was used as a carrier gas and the decomposition rate was 1:30. Results were proportionally expressed for each fatty acid, taking into account the total amount of fatty acids.

B1 (thiamine) analysis:

B1 (thiamine) analysis was performed with a modified HPLC method (HPLC, heating/cooling column, oven column: C 18 OmniSpher 5, 250x4.6 mm Detector: UV 254 nm niacin amide UV 254 nm B1) [30].

2.2 Microbiological Analyses

2.2.1 Sampling and preparation of dilutions

The microbiologically analysed silage samples were weighted at 10 g in sterile stomacher bags. On each sample, 90 mL of sterile physiological peptone water (0.85% NaCl + 0.1% peptone) (PW) was added. After this, the samples were homogenized in stomachers for 2 minutes. In this manner, the samples were diluted by 1:10 with PW. From each of the homogenized samples, 1 mL was transferred into the test tubes which were previously prepared with 9 mL sterile PW. After the test tube was shaken with the vortex, the dilution series of the samples were decimally prepared at up to 10–8 [31].

2.2.2 Counting of the total aerobic mesophilic bacteria

Plate count agar was used for counting of the total aerobic mesophilic bacteria. For counting the total aerobic mesophilic bacteria, 1 mL samples were transferred to petri plates and 12-15 mL Plate Count Agar (PCA) added on samples and samples carefully mixed according to spread plate method. Petri plates were then left for incubation for 24-48 h at 35ºC. After this, the total aerobic mesophilic bacteria count was performed [31].

2.2.3 Counting of the lactic acid bacteria

Man Rogosa Sharpe (MRS) agar was used for lactic acid bacteria count. The 0.1 mL sample was inoculated on the MRS agar using the spread plate method and plates were incubated at 35 ºC for 48 hours. Typical white colonies featuring a diameter of 1-2 mm, which emerged as a result of the incubation, were counted [31].

2.2.4 Counting of the yeast and moulds

Potato Dextrose Agar was used for the counting of yeast and moulds. After sterilizing the agar, 10% sterile tartaric acid was added and pH was set to 3.5. Samples were prepared using the spread plate method. Plates were incubated at 20-25ºC for 5-7 days and the colony counting was performed [31].

3 Results and Discussion

The pH level at the time of storage of the silage produced is known as a good indicator for the evaluation of the quality of the silage. In this study silage production is made via the acid application method, pH level of silage varied between 3.8 and 4.0 during the study. The quality of the silage may be regarded as fine, as it is below pH 4.5.

In the previous studies, it was reported that there was vitamin B1 loss during the manufacture of silage, but no vitamin B1 loss has been observed in the silages manufactured during this study [11] [12] [8].

The fluctuation of this vitamin in the first minced product and during the following days could not be explained.

While the crude protein content of the silage made of whole fish varied between 47.38 and 44.45% over time, the crude protein content of the silage manufactured of processing wastes varied between 37.20% and 37.40% (Table 1 and 2). When these results are compared with fish meal, it is observed that both have a less content of protein than the ratio of 65% which must be contained by a fish meal of medium quality. The first reason for this is that the processing wastes used in the manufacture of silage have a low protein content and the second reason is thought to be the deterioration of the proteins (certain amino acids) as a result of the oxidation of certain fatty acids due to the manufacture of silage at room temperature despite the use of protective antioxidant substances [23]. As whole fish is primarily a human consumption, it may not be regarded as a raw material in the manufacture of silage. However, all of the fishes that are not suitable for human consumption and the dead fish in the cage enterprises may be used as a raw material. It was though that arginine and tryptophan of basic character among the essential amino acids could not be detected because of the method was not convenience to detect such amino acids. When the state of the herring meal and the processing wastes silage in the 30th day are compared in terms of amino acid composition and the content of essential amino acids, valine, methionine and phenylalaline were found to be much lower when compared to those in the fish meal, threonine and lysine were high, while histidine, isoleucine and leucine were found to be similar (Table 3).

Table 1. Changes in Vitamin B1 (mg x 100 g-1 silage), Protein (g x 100 g-1 dry matter), Amino acid contents (g x 100 g-1 protein) by time of Whole Silage of Pearl Mullet.

	Initial	***30th day***	***60th day***	***90th day***	***150th day***
Vitamin B_1 (mg x 100 g^{-1} silage)	0.158±0.00	0.215±0.00	0.205±0.00	0.208±0.00	0.206±0.00
Protein (g x 100 g^{-1} dry matter) (Nx6.25)	47.38±0.18	47.17±0.12	47.40±0.18	46.40±0.00	44.45±0.21
Amino Acids (g x 100 g^{-1} protein)					
Essential Amino Acids (EAA)					
Histidine (His)	1.75±0.03	2.98±0.02	2.54±0.01	2.65±0.04	2.84±0.01
Isoleucine	3.86±0.02	4.13±0.02	3.74±0.02	4.31±0.01	4.34±0.03
Leucine (Leu)	6.71±0.03	7.23±0.04	6.68±0.05	7.08±0.07	7.16±0.04
Lysine (Lys)	8.15±0.01	9.08±0.02	7.84±0.02	8.72±0.02	8.35±0.04
Methionine (Met)	2.46±0.02	1.42±0.01	1.45±0.03	1.85±0.01	2.17±0.01
Phenylalanine (Phe)	3.46±0.06	4.03±0.03	3.28±0.03	3.64±0.07	3.70±0.02
Threonine (Thr)	4.52±0.02	4.81±0.01	4.29±0.00	4.91±0.02	4.43±0.04
Valine (Val)	4.68±0.02	5.06±0.05	4.64±0.05	4.72±0.02	5.25±0.00
Total EAA (g x 100 g^{-1} protein	**35.59**	**38.74**	**34.46**	**37.88**	**38.24**
Nonessential Amino Acids (NEAA)					
Alanine (Ala)	6.25±0.03	6.32±0.01	5.62±0.03	6.03±0.05	5.45±0.01
Glycine (Gly)	6.52±0.03	6.47±0.02	6.36±0.01	6.29±0.01	6.24±0.01
Proline (Pro)	4.51±0.01	5.05±0.05	4.85±0.05	4.77±0.01	5.06±0.03
Serine (Ser)	4.03±0.04	3.84±0.03	3.32±0.03	3.78±0.00	3.49±0.01
Tyrosine (Tyr)	3.26±0.00	3.50±0.01	3.07±0.01	3.74±0.01	3.77±0.02
Total NEAA (g x 100 g^{-1} protein)	**24.57**	**25.18**	**23.22**	**24.61**	**24.01**
[a]EAA/NEAA	**1.45**	**1.54**	**1.48**	**1.54**	**1.59**
Other Amino Acids (OAA)					
Hydroxyproline (Hyp)	1.53±0.02	1.62±0.01	1,55±0.06	1,57±0.03	1.52±0.01
Glutamine (Glu)	13.57±0.05	14.02±0.03	11.44±0.09	13.24±0.03	13.20±0.01
Asparagine (Asp)	7.56±0.02	8.73±0.05	8.70±0.02	8.02±0.04	8.98±0.03
Total OAA (g/100g protein)	**22.66**	**24.37**	**21.69**	**22.83**	**23.7**
Total Amino Acid Value (g x 100 g^{-1} protein)	**82.82**	**88.29**	**79.37**	**85.32**	**85.95**

Essential amino acid index (Chi et al., 2008). 0.95≤ high quality; 0.86–0.95: good quality; 0.75–0.86: usable; ≤ 0.75: inadequate. ± represents std.

Table 2. Changes in Vitamin B1 (mg x 100 g-1 silage), Protein (g x 100 g-1 dry matter), Amino acid contents (g x 100 g-1 protein) by time of the Silage of Processing Wastes.

	Initial	***30th day***	***60th day***	***90th day***	***150th day***
Vitamin B_1 (mg x100 g^{-1} silage)	0.157±0.00	0.202±0.00	0.244±0.00	0.283±0.00	0.176±0.00
Protein (g x 100 g^{-1} dry matter) (Nx6.25)	37.20±0.60	36.14±0.39	38.54±0.18	37.75±0.09	37.40±0.36
Amino Acids (g x 100 g^{-1} protein)					
Essential Amino Acids (EAA)					
Histidine (His)	2.53±0.01	2.28±0.00	2.34±0.00	2.18±0.01	2.22±0.03
Isoleucine	3.80±0.01	3.91±0.01	4.01±0.02	3.84±0.01	4.08±0.02
Leucine (Leu)	6.94±0.04	7.05±0.01	7.29±0.05	6.73±0.00	6.63±0.02
Lysine (Lys)	8.92±0.01	8.33±0.01	9.41±0.04	8.44±0.05	8.68±0.03
Methionine (Met)	1.61±0.00	2.13±0.01	1.86±0.02	1.53±0.03	1.95±0.05
Phenylalanine (Phe)	3.47±0.05	3.70±0.07	4.31±0.01	3.53±0.02	3.42±0.05
Threonine (Thr)	4.62±0.02	4.91±0.05	4.86±0.00	4.75±0.01	5.04±0.02

Valine (Val)	5.04±0.02	5.13±0.01	5.23±0.02	4.90±0.05	4.75±0.00
Total EAA (g x 100 g^{-1} protein)	36.93	37.44	39.31	35.90	36.77
Nonessential Amino Acids (NEAA)					
Alanine (Ala)	5.88±0.01	6.25±0.01	5.97±0.01	5.94±0.01	6.34±0.02
Glycine (Gly)	6.48±0.01	6.78±0.06	5.79±0.00	5.89±0.05	7.99±0.06
Proline (Pro)	5.03±0.12	5.71±0.01	4.86±0.03	4.96±0.04	5.88±0.06
Serine (Ser)	3.89±0.02	4.35±0.04	4.16±0.02	4.20±0.01	4.57±0.04
Tyrosine (Tyr)	3.26±0.02	3.43±0.00	3.57±0.03	3.38±0.04	3.26±0.05
Total NEAA (g x 100 g^{-1} protein)	24.54	26.52	24.35	24.37	28.04
[a]EAA/NEAA	1.51	1.41	1.61	1.47	1.31
Other Amino Acids (OAA)					
Hydroxyproline (Hyp)	1.66±0.11	2.04±0.02	1.44±0.01	1.54±0.00	2.25±0.05
Glutamine (Glu)	13.41±0.02	13.85±0.03	14.03±0.04	12.90±0.02	11.72±0.06
Asparagine (Asp)	8.35±0.05	8.66±0.02	8.25±0.02	8.49±0.06	7.60±0.02
Total OAA (g x 100 g^{-1} protein)	23.42	24.55	23.72	22.93	21.57
Total Amino Acid Value (g x 100 g^{-1} protein)	84.89	88.51	87.38	83.2	86.38

Essential amino acid index [25]. 0.95≤ high quality; 0.86–0.95: good quality; 0.75–0.86: usable; ≤ 0.75: inadequate. ± represents std.

Table 3. Comparison of the essential amino acid of the silages with reference to herring meal

	Whole pearl mullet silage		*Fisheries processing waste silage*		*Herring meal**
Amino Acids (g x100 g^{-1} protein)	*30th day*	*150th day*	*30th day*	*150th day*	
Valine (Val)	5.06	5.25	5.13	4.75	6.00
Leucine (Leu)	7.23	7.16	7.05	6.63	7.20
Isoleucine	4.13	4.34	3.91	4.08	4.13
Threonine (Thr)	4.81	4.43	4.91	5.04	4.00
Methionine (Met)	1.42	2.17	2.13	1.95	3.40
Phenylalanine (Phe)	4.03	3.70	3.70	3.42	5.40
Lysine (Lys)	9.08	8.35	8.33	8.68	7.50
Histidine (His)	2.98	2.84	2.28	2.22	2.03
Arginine	-	-	-	-	6.40
Tryptophan	-	-	-	-	1.1

* [32]

As the amino acid index in the study was above 0.95 for both of the silages (Table 1 and 2), it was concluded that the silages contain high quality protein [25]. It is believed that the silage will be a raw material with high quality protein that could be an alternative to the fish meal when the amount of protein deficit of the silage is supplemented by enrichers.

At the end of the storage time (on 150th day), while the ratio of crude fat was 25.93% in the whole fish silage, a high ratio of 32.71% was observed in the silage of processing wastes (Table 4 and 5). The fact that fat accumulation is observed most among the internal organs of the fish body [23] and the lack of fish meat in the raw material made of processed wastes have led to the proportionally high amount of fat in the waste silage. The extra amount of fat may be reduced by taking the fat in the silage as the fat to be added to the feed ration.

Table 4. Changes in fatty acid composition over the time of the silage made of whole pearl mullet (%)

Fatty Acids	***Initial***	***30th day***	***60th day***	***90th day***	***150th day***
Crude fat x 100 g^{-1} dry matter	26.79±0.66	26.11±0.30	26.60±0.36	26.76±0.09	25.93±0.24
14:0	3.38±0.04	3.56±0.07	3.79±0.06	3.64±0.03	3.85±0.04
16:0	9.18±0.02	9.81±0.10	9.83±0.13	10.94±0.02	10.19±0.04
18:0	2.20±0.01	2.31±0.16	2.35±0.18	2.49±0.21	2.43±0.17
Σ Other SAT	0.80±0.01	0.82±0.01	0.75±0.01	0.68±0.01	0.78±0.01
ΣSAT	15.54±0.03	16.50±0.01	16.72±0.02	17.74±0.21	17.24±0.16
16:1	15.99±0.06	16.43±0.20	16.89±0.15	16.65±0.00	17.07±0.06
18:1n9c	14.25±0.00	14.60±0.07	14.80±0.07	16.22±0.01	15.23±0.05
Σ Other MUFA	2.45±0.01	2.51±0.02	1.30±0.03	1.26±0.01	1.30±0.02
ΣMUFA	32.69±0.02	33.54±0.18	32.98±0.11	34.13±0.01	33.59±0.03
18:2n6c	2.70±0.00	3.15±0.02	3.21±0.02	3.42±0.06	3.37±0.01
18:3n6	0.61±0.01	0.68±0.01	0.69±0.01	0.55±0.01	0.66±0.00
18:3n3	1.67±0.02	1.81±0.05	1.78±0.04	2.02±0.01	1.79±0.03

22:2	2.05±0.01	1.89±0.00	1.95±0.01	1.49±0.04	1.91±0.01
20:5n3	9.77±0.00	9.52±0.02	9.83±0.01	8.41±0.06	9.65±0.02
22:6n3	6.47±0.06	6.01±0.04	6.29±0.07	7.43±0.08	6.32±0.01
Σ Other PUFA	1.85±0.01	1.87±0.00	1.89±0.01	1.77±0.00	1.85±0.01
ΣPUFA	24.49±0.06	24.23±0.04	24.93±0.01	24.53±0.18	24.87±0.01
Defined	72.72±0.11	74.27±0.13	74.63±0.12	72.40±0.02	75.70±0.13
Undefined	27.29±0.11	25.74±0.13	25.38±0.12	20.61±0.02	24.31±0.13
Σ w-3	8.94±0.08	8.60±0.09	8.86±0.03	10.09±0.08	8.88±0.03
Σ w-6	3.31±0.01	3.82±0.03	3.89±0.03	3.97±0.06	4.03±0.01

Other ΣSAT; 15:0, 17:0, Σ Other MUFA; 14:1, 18:1n9t, 20:1n9, 24:1, Σ Other PUFA; 18:3n6, 20:2, 20:3n3, ± represents std.

Table 5. Changes in fatty acids by time of the silage of the processing wastes (%)

Fatty Acids	***Initial***	***30th day***	***60th day***	***90th day***	***150th day***
Crude fat x 100 g^{-1} dry matter	30.21±1.43	30.64±0.90	31.70±0.30	31.43±0.00	32.71±1.20
14:0	4.52±0.02	3.91±0.04	3.82±0.01	3.82±0.01	3.78±0.01
16:0	10.52±0.06	9.85±0.11	9.62±0.07	9.55±0.00	9.55±0.01
18:0	2.88±0.00	2.46±0.02	2.42±0.01	2.42±0.01	2.39±0.02
Σ Other SAT	0.85±0.01	0.76±0.01	0.73±0.01	0.74±0.00	0.74±0.01
ΣSAT	18.77±0.09	16.97±0.19	16.59±0.08	16.52±0.01	16.45±0.02
16:1	18.75±0.05	17.12±0.17	17.10±0.07	17.15±0.02	17.17±0.03
18:1n9c	16.85±0.01	14.29±0.16	14.39±0.18	14.49±0.04	14.43±0.04
Σ Other MUFA	1.42±0.03	1.26±0.01	1.38±0.01	1.37±0.01	1.35±0.02
ΣMUFA	37.02±0.15	32.66±0.28	32.87±0.28	33.00±0.07	32.95±0.01
18:2n6c	2.90±0.02	2.94±0.02	2.95±0.01	3.09±0.01	2.98±0.01
18:3n6	0.62±0.01	0.71±0.01	0.71±0.01	0.71±0.01	0.72±0.01
18:3n3	1.56±0.01	1.80±0.03	1.78±0.01	1.80±0.01	1.78±0.01
22:2	1.63±0.01	2.01±0.01	2.01±0.03	2.04±0.01	2.04±0.01
20:5n3	7.87±0.11	10.55±0.11	10.61±0.13	10.79±0.00	10.82±0.01
22:6n3	4.36±0.03	6.23±0.09	6.25±0.04	6.34±0.02	6.37±0.06
Σ Other PUFA	1.78±0.01	1.95±0.01	1.93±0.01	1.95±0.01	1.95±0.01
ΣPUFA	20.09±0.16	25.47±0.30	25.53±0.25	25.99±0.04	25.92±0.07
Defined	75.87±0.08	75.09±0.78	74.98±0.60	75.51±0.02	75.32±0.04
Undefined	24.14±0.08	24.91±0.78	25.03±0.60	24.50±0.02	24.69±0.04
Σ w-3	6.67±0.04	8.86±0.13	8.85±0.06	8.97±0.04	8.98±0.06
Σ w-6	3.51±0.03	3.65±0.04	3.66±0.02	3.80±0.01	3.69±0.01

Other ΣSAT; 15:0, 17:0, Σ Other MUFA ; 14:1, 18:1n9t, 20:1n9, 24:1, Σ Other PUFA; 18:3n6, 20:2, 20:3n3, ± represents std.

No significant change was observed in the fatty acid content over time in the silage of whole pearl mullet (Table 4). The highest total of PUFA (Polyunsaturated Fatty Acids) and the total MUFA (Monounsaturated Fatty Acids) content were found to be 24% and 34% respectively. Eicosapentaenoic acid (EPA) was of the highest fatty acid among the PUFA, and it was followed by docosahexaenoic acid (DHA). The content of omega 3 and omega 6 fatty acids were determined as 10.09% and 4.03% respectively. The total saturated fatty acids which were at the level of 15% at the beginning, have increased and reached the level of 17% after the 150th day.

The total PUFA content in the processing waste silages varied over time (Table 5). While the initial content was 20%, it reached the level of 25% as the waiting time increased. EPA,

which has the highest value among the PUFA reached the level of 10% from 7% over time. Similarly, DHA reached from 4% to 6% level. It was detected that the saturated fatty acids decreased from 18% to 16% over time in the processing waste silage. The total MUFA content decreased from %37 to 32% in parallel with the saturated fatty acids.

Differences were determined between the pearl mullet silage and the processing waste silage in terms of the fatty acids. The processing waste silage had the highest fatty acid content with a total of PUFA of 25% and omega 3 of 9%. An increase in the fatty acid content over time was determined in both silages. Among the reasons leading to the increases, especially in the unsaturated fatty acid content is the liberation of the fatty acids as a result of the reactions of the lactic acid bacteria at low pH. Both the raw material is protected and the valuable fatty acids liberate and lead to an increase in their ratio as a result of the bond rupture and transformation of the fat molecules at the stage of silage manufacture using acid. Here, the bacteriological activities may be counted among the major modifiers.

The reproduction of the microorganisms was observed in the period up to the 30th day of the storage of the silages, while no reproduction was determined after the 60th day (Table 6). On the days between the 30th and 60th the microorganism reproduction stopped. But this could not be determined certainly because the long-time intervals of the analysing periods (0th, 7th, 15th, 30th, 60th, 120th days). The fact that mould and lactobacilli did not proliferate on the 7th day in the processing waste silage, and the re-reproduction seen on the 15th day could not be explained. It was reported that the number of bacteria decreased after the acid supplement during the period starting from the beginning until 15th day, however the acid supplement could not prevent the development of mould in long-term storage [32]. Mach and Nortvedt (2009) reported that the colonies of bacteria and mould spores decreased to the lowest level on 30th day, but they increased gradually by the 45th day. It is stated that only colonies of bacteria were observed in the 60th day and this cannot explain the lack of mould reproduction.

Table 6. PCA (Total aerobic bacteria count, cfu x g-1), PDA (yeast and mold count, cfu x g-1) and MRS (lactic acid bacteria count, cfu x g-1) values observed over time in the silage samples produced (cfu x g-1: colony forming unit x g-1)

	Whole pearl mullet silage			Fisheries processing waste silage		
	PCA	PDA	MRS	PCA	PDA	MRS
Raw material*	$8x10^5$	$1.4x10^3$	$1x10^2$	$2.6x10^5$	$5x10^4$	$2x10^4$
7th Day	$2x10^2$	$5x10^2$	$1.5x10^2$	$5x10^1$	-	-
15th Day	$1.4x10^5$	$3.8 x10^4$	$1.1x10^3$	$7.5x10^4$	$2x10^2$	$2x10^2$
30th Day	-	$1x10^1$	$1.8x10^2$	$13.5x10^1$	$6x10^2$	$1.5x10^2$
60th Day	-	-	-	-	-	-
90th Day	-	-	-	-	-	-
120th Day	-	-	-	-	-	-

*Raw material before adding acid
** "-" means there is no reproduction

4 Conclusions

As a result of this study, it was concluded that silage may also be used right after it maturates or after some storage, as the total amount of organisms determined during the period after the acid treatment process is below the upper limit indicated in the legislation.

In this study, it was shown that the silage manufactured from processing industrial wastes can be a raw material that could be an alternative to the fish meal when the amount of protein deficit of the silage is supplemented by enrichers. The amino acid deficit which is of low amount in the silage when compared to the fish meal can be supplemented by adding these amino acids externally to the silage. The product obtained with the manufacture of its silage has a high content of free amino acids and fatty acids may bring a competitive advantage against fish meal. It was seen that there is no microbiologically problem, like an increase in the number of bacteria or another negative change in microbiological quality parameters, to use the produced silage immediately after its maturity or storage. The processing waste silage produced in this research can be an alternative feed raw material to the fish feed industry and the factories producing pet food.

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