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Research Article

Production of amino acid-rich fertilizer from sturgeon waste biosilage

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Abstract

Fish processing generates substantial amounts of organic waste, particularly from high-value species like sturgeon. Transforming these nutrient-rich wastes into value-added products, such as biosilage and amino acid fertilizers, offers an environmentally friendly solution that aligns with the principles of a circular bioeconomy. This study aimed to produce biosilage from sturgeon waste through microbial fermentation and to evaluate its potential for amino acid fertilizer production. Initially, biosilage was prepared and subjected to quality control through assessments of proximate composition and microbial indices. Molecular identification of the dominant fermentative bacteria using 16S rRNA sequencing revealed the presence of *Bacillus cereus* (99.5% similarity; Accession No. KP940382.1), as well as lactic acid bacteria, including *Lactobacillus plantarum, L. acidophilus, and L. pentosus*. The biosilage demonstrated significantly higher levels of protein (67.7%), lipid (14.06%), and ash (11.26%), along with lower moisture content (6.98%) compared to raw waste (p<0.05). Microbial analysis confirmed the safety of the biosilage, showing high levels of lactic acid bacteria (6.48 log CFU/g) and the absence of *Escherichia coli* and *Salmonella typhimurium*. After quality control, an amino acid fertilizer was produced from the biosilage. The amino acid profile revealed total and free amino acid contents of 14.02% and 8.08%, respectively, with glutamic acid and leucine being the most abundant. Additionally, elemental analysis showed a balanced nutrient content, including total nitrogen (3.56%), phosphorus (0.81%), potassium (0.34%), and organic matter (17.92%). In conclusion, the stepwise conversion of sturgeon waste into biosilage and amino acid fertilizer represents eco-friendly strategy for waste valorization.

Introduction

The global fish processing industry generates millions of tons of waste annually, including heads, skins, bones, and viscera, which, if not managed properly, can pose serious environmental and economic concerns (Rustad *et al*., 2011). Sturgeon processing, in particular, leads to considerable volumes of such by-products due to the high value of caviar, while the remaining biomass often goes underutilized. Considering the production of more than 4700 tons of sturgeon in Iran in 2023, if the expected waste percentage considered about 35%-50% on average, the total waste produced will be approximately 1600-2300 tons (Iranian Fisheries Statistical Yearbook, 2024). Sturgeon (caviar-processing) waste was specifically selected because it is generated in large quantities, has exceptionally high protein content, and currently lacks efficient valorization pathways. Unlike many other fish wastes, sturgeon by-products contain stable collagen-rich proteins and valuable nitrogenous compounds, making them an excellent substrate for producing amino-acid fertilizers. Moreover, the aquaculture expansion of sturgeon species has increased the volume of underutilized waste, creating both an environmental challenge and an opportunity for converting this resource into high-value agricultural inputs. Bioconversion of these residues into fish silage via enzymatic or microbial fermentation has been increasingly studied as a sustainable waste valorization strategy (Shahidi and Synowiecki, 1997; Arruda *et al*., 2007; Sarhadi *et al.,* 2012).

Fish silage is a fermented product that can be produced via two main methodologies: acid-based and biological. Various types of fish waste, including those from warm-water, cold-water, tuna, and shrimp, can be utilized for silage production (Peñarubia *et al*., 2020). Biological silage, or biosilage, can be produced through two primary techniques: autolysis using endogenous enzymes or fermentation using microbial inoculants (Marti-Quijal *et al*., 2020). Common microbial starters include lactic acid bacteria, spore-forming Gram-positive bacteria (e.g., *Bacillus* spp.), and a variety of yeasts (Ahmed and Mahendrakar, 1996). Fish silage, characterized by its high content of soluble proteins, peptides, and free amino acids, offers promising applications in agriculture as a base material for organic fertilizers (Peñarubia *et al*., 2020; Radziemska *et al.,* 2019). Its high content of essential nutrients—particularly nitrogen, phosphorus, and potassium—contributes to plant growth, enhances crop quality, and increases yields (Ahuja *et al*., 2020). This enhanced microbial diversity can lead to more sustainable soil health (Karim *et al*., 2015).

Several studies have demonstrated the potential of amino acid-based fertilizers derived from fish waste silage in enhancing plant growth and nutrient uptake. For example, Wang *et al*. (2023) reported that liquid biofertilizers produced from fermented fish waste significantly increased nitrogen availability in soils. Similarly, Al-Malieky and Jerry (2019) developed a hydrolyzed fish waste that improved crop yields in saline soils. Moreover, Madende and Hayes (2020) highlighted the bio-stimulant effects of amino acid-rich silage extracts on root

development and chlorophyll content in horticultural crops. The environmental benefits of using fish silage are equally significant. As it decomposes, it enriches soil organic matter, enhances moisture retention, and reduces reliance on synthetic fertilizers—thereby lowering the environmental footprint of agricultural practices. The utilization of fish silage thus aligns with sustainable agriculture goals and efficient resource use in both agricultural and aquaculture systems (Ilera-Vives *et al*., 2013). A more recent line of research further supports these conclusions: In 2024, Mahdavi et al. demonstrated that applying a bio-fertilizer derived from fish waste to Stevia rebaudiana under salinity stress significantly improved growth parameters, photosynthetic pigment levels, and water content — while reducing oxidative stress markers at high salinity (Mahdavi *et al*., 2024). In another recent study, researchers produced hydrolysates from fish waste that acted as effective plant biostimulants, improving nutrient uptake and promoting healthier growth when added to soils (Nuzhyna *et al*., 2025). Moreover, a comprehensive life-cycle assessment published in 2025 showed that converting marine fish-head waste into nitrogen-based nutrients (e.g., peptones) leads to substantially lower greenhouse-gas emissions compared with conventional land-based nitrogen sources — with up to ~88% reduction in CO_2-equivalents (Lee *et al*., 2025).

The use of biosilage derived from sturgeon caviar-processing waste for producing amino-acid fertilizers has not been adequately investigated, and the behavior of this material under enzymatic hydrolysis remains largely unexplored. Moreover, the composition, quality, and agricultural potential of the resulting amino-acid fertilizer have not been sufficiently documented in previous studies, creating a clear research gap. Amino-acid fertilizer was selected instead of fishmeal or compost because enzymatically hydrolyzed amino acids provide faster nutrient availability, higher plant uptake efficiency, and improved bio-stimulant effects. Unlike fishmeal and compost, which rely on slow microbial degradation, amino-acid fertilizers deliver readily absorbable nitrogen and bioactive peptides directly to plants. Moreover, converting sturgeon waste into amino-acid fertilizer generates a higher-value product with broader agricultural applications, while also reducing odor, pathogen load, and processing time compared to composting or fishmeal production (Colla *et al.,* 2015).

The production of amino acid fertilizers from sturgeon fish waste not only contributes to the circular bioeconomy by converting waste into value-added products but also provides an environmentally friendly alternative to synthetic fertilizers. This study investigates the formulation and characterization of amino acid fertilizer derived from biosilage made from sturgeon by-products, aiming to assess its chemical properties and potential agricultural benefits.

Materials and methods

Preparation of sturgeon waste and biosilage production process

Sturgeon waste (viscera) was obtained from the Islami Sturgeon Farming Center and

promptly transported under cold chain conditions to the processing unit at the Caspian Sea Ecology Research Center (CSERC). Upon arrival, the waste was stored at –20°C until further use (after 48 hours). Prior to processing, the samples were thawed, minced, and transferred to a stainless-steel fermenter with a capacity of one ton. The fermenter temperature was maintained at 45°C to preserve the activity of endogenous enzymes (Marti-Quijal *et al*., 2020). In the subsequent phase, a combination of proteolytic microorganisms including spore-forming gram-positive bacteria capable of producing proteases and lactic acid-producing bacteria (*Lactobacillus plantarum* PTCC 1745, *Lactobacillus acidophilus* PTCC 1643, *Lactobacillus pentosus* PTCC 1898) were introduced to facilitate biodegradation. A 5% (v/v) inoculum of a bacterial consortium at a concentration of 10^8 CFU/mL (log 8) was added to the medium (pH= 5.5-6.5). During bacterial inoculation, sugarcane molasses was added as a carbohydrate source at a concentration of 15%. The samples were incubated in a fermenter at a temperature of 40–45°C under shaking conditions for 48 hours (The pH was measured at specific intervals and adjusted to a range of 5.5 to 6.5). Upon completion of the process, the lipid layer was collected from the surface of the hydrolyzed solution using a stainless steel surface skimmer (Aban Arya Oil Skimmer). The hydrolyzed solution was then concentrated using a vacuum evaporator (Heidolph Hei-VAP Industrial) under reduced pressure. Subsequently, the concentrated material was dried using a vacuum dryer (Büchi Lyovapor L-200) at 55–60°C for 24 hours. In the final stage, the dried samples were milled using a grinding device to obtain particles with a mesh size of less than 1 mm and subsequently packaged (Safari *et al*., 2020; 2021).

Proximate composition of biosilage

Moisture content was determined by drying the sample in a hot-air oven at 105°C until a constant weight was achieved. Ash content was analyzed using a muffle furnace at 550°C. Crude protein was measured using the Kjeldahl method, while lipid content was determined via Soxhlet extraction (AOAC, 2019).

Microbiological analysis of biosilage

To determine the total viable bacterial count in biosilage samples, Tryptic Soy Agar (TSA; Merck, Germany) was utilized as a non-selective culture medium. Serial ten-fold dilutions of each sample were prepared in sterile physiological saline (0.85% NaCl), and 0.1 ml aliquots were surface-plated on TSA in triplicate. When necessary, additional dilutions (up to 10^{-6}) were prepared to ensure countable colonies (30–300 CFU per plate). Plates were incubated aerobically at 35±2°C for 48 hours, and the colony-forming units (CFU) were counted to determine the total bacterial load, following the method described by ISO 4833-1, 2013. For the enumeration of coliforms, fecal coliforms, and Escherichia coli, CHROMagar™ ECC (CHROMagar, France) was employed. A 0.1 ml aliquot of the appropriate dilutions was spread on the medium and incubated at 35°C and 44.5°C for 24–48 hours. The appearance of dark blue to violet colonies at both temperatures indicated E. coli, while

the presence of pink to red colonies at 35°C indicated total coliforms, and their growth at 44.5°C confirmed fecal coliforms (ISO 9308-1, 2014; CHROMagar, 2021). For the quantification of lactic acid bacteria (LAB), de Man, Rogosa and Sharpe (MRS) agar (Oxoid, UK) was used. Diluted samples (0.1 ml) were surface-inoculated on MRS plates and incubated anaerobically at 30±1°C for 48–72 hours. Anaerobic conditions were achieved using GasPak™ EZ Anaerobe Container System (BD, USA) in sealed jars. Colony counts were conducted in accordance with ISO 15214, 1998. Fungal and yeast populations were assessed using acidified Potato Dextrose Agar (PDA; Merck, Germany), adjusted to pH 3.5±0.1 with sterile tartaric acid to suppress bacterial growth. After plating 0.1 ml of serial dilutions on PDA, the plates were incubated aerobically at 22–25°C for 5–7 days. Colony counts were interpreted based on ISO 21527-1, 2008.

Molecular identification of spore-forming gram-positive bacteria

The molecular identification of spore-forming gram-positive bacteria was performed using polymerase chain reaction (PCR) amplification and sequencing of the 16S rRNA gene. Bacterial isolates were first cultured on nutrient agar at 37°C for 24 hours to ensure pure colony formation. A single colony from each isolate was selected, and genomic DNA was extracted using a commercial DNA extraction kit (Qiagen, Germany) following the manufacturer's instructions. PCR amplification of the 16S rRNA gene was carried out using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). Each 25 µL PCR reaction contained 12.5 µL of PCR Master Mix (Thermo Fisher Scientific, USA), 1 µL of each primer (10 µM), 2 µL of DNA template, and 8.5 µL of nuclease-free water. The thermal cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. PCR products were visualized by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and observed under UV illumination. Successful amplification resulted in a product of approximately 1500 bp. The PCR products were purified using a PCR purification kit (Qiagen, Germany) and sequenced bi-directionally using the Sanger method. The obtained sequences were edited and assembled using BioEdit software and then compared against the NCBI GenBank database using the BLAST algorithm (Woo *et al*., 2008).

Preparation of amino acid fertilizer

Based on the proximate analysis of biosilage derived from sturgeon waste and the determination of its protein content, a suspension was prepared by dissolving 200 g of dried biosilage in 500 ml of distilled water. After initial shaking and pH adjustment, the final volume was brought to 1000 ml. The formulated product was subsequently analyzed for its total amino acid profile, free amino acid content, as well as inorganic salts and organic matter (Moore 2004; Zhang *et al*., 2017).

Amino acid profiles of the fertilizer

After defrosting, liquid amino acid fertilizer samples were homogenized thoroughly. High-performance liquid chromatography (HPLC) was employed to quantify and identify amino acids, using a Cecil Adept HPLC System (Series 9000, UK) equipped with a quaternary gradient pump, a UV-Vis detector set at 338 nm, and a C18 reversed-phase column (250 mm×4.6 mm, 5 µm particle size). The system was operated using Power Stream software for data acquisition and analysis (Moore, 2004; ISO 13903, 2005).

Sample preparation was performed in two steps:

- Hydrolysis: For total amino acid analysis, 1 mL of the liquid fertilizer sample was hydrolyzed with 6N hydrochloric acid (HCl) in sealed glass ampoules at 110°C for 24 hours under a nitrogen atmosphere to prevent oxidation.
- Derivatization: Post-hydrolysis, samples were neutralized, filtered, and derivatized with orthophthalaldehyde (OPA) reagent, following the method of Henderson *et al*. (2000), to enable UV detection.

For free amino acid analysis, hydrolysis was omitted to preserve thermolabile amino acids. After derivatization, the samples and amino acid standards were dried in a vacuum oven at 40°C, followed by the addition of 200 µL of a suitable diluent (sodium acetate buffer/methanol). Tubes were vortexed gently and centrifuged at 6200 g for 5 minutes. Finally, 20 µL of the clear supernatant was injected into the HPLC system. Quantification was achieved by comparing peak areas and retention times with certified amino acid standards. Each sample was analyzed in triplicate to ensure reproducibility.

Mineral content, total nitrogen, and organic matter of the fertilizer

To determine the mineral composition of fertilizer derived from biosilage, samples were initially oven-dried at 80°C to constant weight. Approximately 0.3 g of each dried sample was digested with 4 ml of concentrated nitric acid (HNO_3, ≥65%) at ambient temperature for 1 hour. The digestion process was completed by heating the mixture on a hot plate at 90°C for 3 hours until complete decomposition of the organic matrix. Once cooled, the digested mixture was diluted to 50 mL with deionized water, and 1 mL of 2% potassium bichromate solution was added to stabilize trace metal ions. Mineral analysis was conducted using a flame atomic absorption spectrophotometer (AAS; Thermo Scientific iCE 3000 Series, USA) equipped with element-specific hollow cathode lamps. Potassium (K), calcium (Ca), manganese (Mn), and phosphorus (P) were quantified using the flame technique, and the results were obtained by constructing calibration curves via the standard addition method. All data acquisition and processing were performed using WinLab32 software. The methodology followed the protocols outlined by the American Public Health Association (APHA, 2017) and standardized by the U.S. Environmental Protection Agency (USEPA, 2007).

For the determination of total nitrogen (N), the Kjeldahl method was employed. Samples were digested with concentrated sulfuric acid in the presence of a catalyst to

convert organic nitrogen into ammonium sulfate. The digested solution was then alkalized and distilled, converting ammonium ions into free ammonia. The ammonia was absorbed in boric acid, forming ammonium borate, which was subsequently titrated with 1.0 N sulfuric acid. The volume of acid consumed was used to calculate the total nitrogen content, in accordance with AOAC Official Method 978.04 (AOAC, 2019).

The organic matter content was determined using the Walkley-Black method, a wet oxidation technique. In this method, the sample was treated with a known volume of concentrated sulfuric acid and potassium dichromate. After complete oxidation, the excess dichromate was titrated with standardized ferrous ammonium sulfate solution. The organic carbon content was calculated based on the amount of dichromate reduced, and converted to organic matter using a correction factor (ISO 10694:1995).

Statistical analysis

This study used a completely randomized design (CRD) with three replications for each treatment and control. Data analysis was performed using SPSS 22 software and one-way analysis of variance. Comparison of means between different treatments was determined based on t- test at a 5% probability level (p<0.05).

Results

Molecular identification of spore-forming gram-positive bacteria

Following PCR amplification and sequencing of the 16S rRNA gene, high-quality sequences approximately 1500 bp in length were obtained from the unknown bacterial isolate. BLAST analysis against the NCBI GenBank database revealed that the 16S rRNA gene sequence shared 99.5% similarity with *Bacillus cereus* reference strains. Further analysis of the guanine-cytosine (G+C) content of the obtained sequence showed a high G+C percentage (approximately 35%), which is consistent with reported values for *Bacillus cereus* species (35-37% G+C content). The combination of high sequence similarity and G+C content supported the identification of the unknown spore-forming Gram-positive bacterium as *Bacillus cereus* (Accession number: KP940382.1). To address safety considerations, we performed an in vivo assessment by intraperitoneally injecting fish with a dose of 10^8 CFU per fish. No negative effects or mortality were observed during the experimental period, suggesting that under these experimental conditions, the strain did not cause acute pathogenicity. We acknowledge that no toxin gene testing or formal risk assessment was performed; therefore, caution should be exercised in interpreting these results.

Proximate composition

The results showed that for every 5 kg of raw primary waste, one kg of dry biosilage with a protein content of 67% was produced. Table 1 shows the chemical composition of sturgeon waste and biosilage. According to the table, the amount of protein, fat and ash in biosilage was higher than that of waste (p<0.05). However, the percentage of moisture in waste was higher than that of biosilage (p<0.05).

Microbiological analysis

Table 2 shows the results of microbial indices, including total bacterial count, yeast and mold, lactic acid bacteria, coliforms, non-*Escherichia coli* fecal coliforms, *E. coli*, and *Salmonella typhimurium*. The microbial indices, expressed in logarithms of counts per gram, were 8.63, 3.17, 6.48, 1.45, <1, <1, and not detected, respectively.

Table 1: Proximate composition of raw sturgeon waste and prepared biosilage (wet weight).

Proximate factors (%)	Raw waste	Biosilage
Protein	18.26±0.51[b]	67.7±1.14[a]
Lipid	8.45±0.23[b]	14.06±0.18[a]
Moisture	68.45±1.12[a]	6.98±0.57[b]
Ash	5.06±0.14[b]	11.26±0.45[a]

Non-identical letters in each row indicate a significant difference at the 5% level (p<0.05).

Table 2: Microbial indicators (Log CFU/g) in biosilage produced from sturgeon waste.

Indicator	(Log CFU/g)
Total viable count	8.63±0.50
Yeast and mold	3.17±0.06
Lactic acid bacteria	6.48±0.10
Total coliforms	1.45±0.1
Fecal coliforms (non *E. coli*)	<1
E. coli	<1
Detection of *S. typhimurium*	Not detected

Amino acid profile

Results of amino acid profile in amino acid fertilizer prepared from sturgeon waste biosilage are shown in Table 3. Among the essential and non-essential amino acids, leucine and glutamic acid were found to have the highest concentrations, with 15.25 and 31.04 mg AA/g sample, respectively. The percentage of total amino acids and free amino acids were 14.02 and 8.08, respectively, and the percentage of free amino acids in the final product is 57.63%, and it can be used as an amino acid fertilizer with a high amino acid percentage.

Table 3: Total and free amino acids composition (mg AA/g sample) in amino acid fertilizer prepared from sturgeon waste biosilage .

Amino acid	Total amino acids	Free amino acids
Histidine*	2.44±0.01[a]	1.79±0.01[b]
Isoleucine *	6.55±0.11[a]	4.85±0.02[b]
Leucine *	15.25±0.3[a]	10.78±0.03[b]
Lysine *	12.17±0.31[a]	9.22±0.01b[b]
Methionine *	3.71±0.02[a]	3.67±0.02[a]
Phenyl alanine *	5.91±0.01[a]	4.56±0.02[b]
Tyrosine [b]	3.45±0.01[a]	3.37±0.01[a]
Threonine *	4.75±0.02[a]	1.35±0.01[b]

Table 3 continued:		
Arginine *	4.56±0.05[a]	1.65±0.01[b]
Valine *	9.74±0.21[a]	5.86±0.03[b]
Aspartic acid [b]	11.62±0.41[a]	5.11±0.02[b]
Glycine [b]	6.75±0.05[a]	1.72±0.01[b]
Alanine [b]	7.36±0.02[a]	3.46±0.01[b]
Serine [b]	5.53±0.14[a]	4.57±0.02[b]
Glutamic acid [b]	31.04±0.65[a]	14.36±0.3[b]
Proline [b]	9.25±0.06[a]	4.45±0.02[b]
Cysteine [b]	0.14±0.01[a]	0.12±0.01[b]
Sum of amino acids (W/W%)	14.02	8.08

Non-identical letters in each row indicate a significant difference at the 5% level (n=3; $p<0.05$).
* Essential amino acids.

Mineral content, total nitrogen, and organic matter

Total nitrogen, total phosphorus, dry matter, ash percentage, and trace elements including magnesium, calcium, potassium, and organic matter content were analyzed, with the measured values presented in Table 4.

Table 4: Analysis of important elements (%) in amino acid fertilizer prepared from sturgeon waste biosilage (n=3).

Factors (%)	Results
Total nitrogen (%)	3.56±0.07
Organic nitrogen	2.31±0.05
Inorganic nitrogen	1.25±0.02
Total phosphorus (%)	0.81±0.03
Magnesium (%)	0.02±0.003
Organic matter (%)	17.92±1.16
Calcium (%)	0.03±0.001
Potassium (%)	0.34±0.02
Dry matter (%)	21.11±0.61

Discussion

The proximate analysis revealed significant improvement in the nutritional composition of biosilage compared to raw sturgeon waste. Protein content increased from 18.26% to 67.7%, indicating effective hydrolysis of complex proteins into simpler forms during fermentation. Similar increases in protein reported by Shobihah *et al*. (2025), who observed a rise in protein content in fermented fish waste due to microbial protease activity. This makes the biosilage a valuable protein source for agricultural and feed applications. Lipid content also increased significantly (from 8.45% to 14.06%), likely due to microbial release of bound lipids during breakdown. Moisture content dropped markedly (from 68.45% to 6.98%), which is beneficial for storage and shelf stability, similar to findings by Shobihah *et al*. (2025). Ash content, an indicator of mineral concentration, more than doubled (5.06% to 11.26%), suggesting enrichment in inorganic nutrients during biosilage processing.

The microbiological analysis indicated a high total viable count (8.63 log CFU/g), which is typical in fermented products due to microbial proliferation. Lactic acid bacteria (6.48 log CFU/g) dominated the beneficial flora, which is consistent with successful silage fermentation and aligns with the findings of Lee *et al*. (2016), who noted lactic acid bacteria as dominant in biosilage from seafood waste. Importantly, pathogenic indicators such as *E. coli*, fecal

coliforms, and *Salmonella typhimurium* were below detection limits, confirming microbial safety. These results compare favorably with studies by Raeesi *et al*. (2023), who reported successful pathogen suppression during anaerobic biosilage fermentation of fish by-products. When a combination of *Bacillus cereus* and lactic acid bacteria is used for producing biosilage from sturgeon fish waste, the total bacterial population increases by the end of the fermentation process. Notably, Bacillus shows a higher proliferation compared to lactic acid bacteria. This can be attributed to several factors: B. cereus is a spore-forming bacterium with high resistance to environmental stresses such as pH fluctuations, oxygen levels, and nutrient limitations commonly present in fish waste. Moreover, its metabolic versatility allows it to utilize a broader range of proteins and amino acids released during fish tissue breakdown, providing a growth advantage over lactic acid bacteria, which primarily rely on carbohydrate fermentation. Additionally, the inoculated Bacillus may produce extracellular enzymes that further enhance nutrient availability, indirectly supporting its own growth and leading to a higher final population (Esakkiraj *et al.,* 2016).

Agricultural fertilizers produced from fish waste are one of the rich and sustainable sources of amino acids, providing essential nutrients for plants and possessing unique characteristics (Mehta *et al*., 2023; Liu *et al*., 2016). Amino acids are essential components of proteins, and their provision through this fertilizer can help improve plant growth and health (Ahuja *et al*., 2020). The amino acid fertilizer derived from sturgeon waste biosilage showed a notably high concentration of both total and free amino acids. The dominance of glutamic acid (31.04 mg/g) and leucine (15.25 mg/g) not only reflects the protein-rich origin of the raw material but also underscores the bioconversion efficiency of the fermentation process. The observed values were higher than those reported by Zhang *et al*. (2018) in fish waste hydrolysates (glutamic acid: ~22 mg/g), indicating the superior nutritional quality of sturgeon-based biosilage. Glutamic acid is central to ammonium assimilation through the glutamine synthetase-glutamate synthase (GS-GOGAT) cycle, which directly influences plant nitrogen metabolism and chlorophyll biosynthesis (Forde and Lea, 2007; Xu *et al*., 2021).

Free amino acid content (8.08%) and its share in total amino acids (14.02%) make this fertilizer particularly effective for foliar application, where quick absorption is crucial. Similar trends were noted by Heidarzadeh (2025), who highlighted the efficiency of free amino acids in promoting plant growth and resistance. Free amino acids especially lysine, proline, and glutamic acid—are known to act as osmoprotectants under stress conditions such as salinity or drought, improving plant tolerance through antioxidant enzyme regulation (Ali *et al*., 2019). Leucine, isoleucine, and valine (branched-chain amino acids) have also been associated with signaling pathways in root architecture and nutrient sensing via the TOR (Target of Rapamycin) cascade (Dong *et al*., 2017). These physiological roles make free amino acid-based fertilizers effective biostimulants, not merely nutrient sources.

In terms of elemental composition, the amino acid fertilizer showed a balanced nutrient profile. The total nitrogen content (3.56%), largely in organic form (2.31%), indicates a sustainable and eco-friendly nutrient release profile. Organic nitrogen reduces nitrate leaching risks and supports long-term microbial biomass buildup in soil (Gaskell and Smith, 2007). Moreover, amino acid-bound nitrogen is more efficiently utilized by plants compared to inorganic forms, especially in calcareous or sandy soils (Calvo *et al*., 2014; Wang *et al*., 2025). The phosphorus level (0.81%) is adequate for promoting root development, flowering, and energy transfer via ATP synthesis. Total phosphorus (0.81%) was comparable to values reported by Shenbagavalli *et al*. (2020) in organic fertilizers derived from fish waste (~0.8%). Compared to traditional organic fertilizers like compost or vermicompost—often containing <0.5% P—this formulation provides superior phosphorus availability (Shenbagavalli *et al*., 2020). The organic matter content (17.92%) significantly contributes to improved soil structure, water holding capacity, and nutrient retention. It also enhances cation exchange capacity (CEC), facilitating better availability of calcium, potassium, and magnesium (Lal, 2020). Although the calcium (0.03%) and magnesium (0.02%) contents are modest, their bioavailability from organic matrices is often higher than inorganic salts due to complexation with amino acids (White and Broadley, 2003). Calcium plays a crucial role in cell wall integrity and root hair development, while magnesium is the core atom in chlorophyll and activates many photosynthetic enzymes. Potassium (0.34%) is essential for stomatal function, enzyme activation, and resistance against abiotic stress. Its level in this fertilizer matches or exceeds that found in many fish-based fertilizers reported in previous studies (Zhang *et al*., 2018). These concentrations are also within acceptable ranges for biostimulant products and support plant vigor and resilience (Marschner, 2012).

Limitations and Potential Solutions for Using Sturgeon Fish Waste

1- High spoilage risk: Fish waste is highly perishable due to its rich protein and lipid content. Rapid processing, immediate cooling or freezing, and controlled fermentation (biosilage) can minimize spoilage.
2- Presence of pathogens and toxins: Fish waste may harbor pathogenic bacteria or biogenic amines. Use of safe inoculated strains, adherence to biosafety guidelines (BSL-1 or BSL-2), and regular microbial and chemical monitoring of the final product.
3- High lipid content and oxidation: Polyunsaturated fatty acids in fish waste are prone to oxidation, reducing product quality. Addition of antioxidants, temperature control, and proper packaging to minimize oxygen exposure.
4- Variability in composition: Protein, lipid, and mineral content can vary between seasons and individual fish. Sampling and compositional analysis of the waste prior to processing, with adjustment of ratios to ensure process consistency.

5- Environmental concerns: Improper disposal may lead to nutrient pollution and eutrophication. Valorization of fish waste through biosilage production or other nutrient-recycling processes instead of direct disposal.
6- Economic and logistical challenges: Collection, storage, and transport require cold-chain management. Establish organized collection networks, use appropriate cooling equipment, and minimize the time between waste generation and processing.

In conclusion, the amino acid fertilizer derived from sturgeon waste biosilage offers a safe, cost-effective (An economic analysis conducted on the production of biosilage from sturgeon waste and its comparison with the production of fish meal shows that the cost price of biosilage is half that of fish meal and therefore has economic justification. It should be noted that economic justification is not shown in this study), and environmentally friendly alternative to synthetic fertilizers. With its dual role as a nutrient source and biostimulant, this organic formulation holds strong potential for enhancing crop yield, plant health, and sustainable agriculture practices. Its high free amino acid content ensures rapid absorption and utilization by plants, while slow-releasing organic nitrogen and minerals improve long-term soil fertility. Thus, sturgeon waste biosilage presents a sustainable and efficient solution for organic fertilization, aligning with circular economy principles and environmental stewardship.

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References

Ahmed, J. and Mahendrakar, N.S., 1996. Autolysis and rancidity development in tropical freshwater fish viscera during fermentation. *Bioresource Technology*, 58, 247–251. DOI:10.1016/S0960-8524(96)00085-5

Ahuja, I., Dauksas, E., Remme, J.F., Richardsen, R. and Løes, A.K., 2020. Fish and fish waste-based fertilizers in organic farming – With status in Norway: A review. *Waste Management*, 115, 95–112. DOI:10.1016/j.wasman.2020.07.025

Ali, Q., Ahsan, H. and Farooq, M.A., 2019. Role of amino acids in stress tolerance in plants. *Plant Physiology Reports*, 24, 402–412. DOI:10.1201/9780203705315-12

Al-Malieky, H.M. and Jerry, A.N., 2019. Preparation Protein Hydrolysates from Fish by-product and Study Effected on Lettuce (*Lactuca sativa* L.) Growth, Yield, Quality and Enhanced Salt Tolerance. *Basrah Journal of Agricultural Sciences,* 32, 246–255.

AOAC, 2019. Official Methods of Analysis, Association of Official Analytical Chemists International, 4th ed. Arlington, VA, USA.

APHA, 2017. Standard Methods for the Examination of Water and Wastewater, 23rd ed. American Public Health Association, Washington DC, USA.

Arruda, L.F.D., Borghesi, R. and Oetterer, M., 2007. Use of fish waste as silage: a review. *Brazilian Archives of Biology and Technology*, 50, 879–886. DOI:10.1590/S1516-89132007000500016.

Arvanitoyannis, I.S. and Kassaveti, A., 2008. Fish industry waste: treatments, environmental impacts, current and potential uses. *International Journal of Food Science & Technology*, 43, 726–745. DOI:10.1111/j.1365-2621.2006.01513.x

Atelge, M.R., Krisa, D., Kumar, G., Eskicioglu, C., Nguyen, D.D., Chang, S.W. and Unalan, S., 2020. Biogas production from organic waste: recent progress and perspectives. *Waste and Biomass Valorization*, 11, 1019–1040. DOI:10.1007/s12649-018-00546-0

Calvo, P., Nelson, L. and Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. *Plant and Soil*, 383, 3–41. DOI 10.1007/s11104-014-2131-8

CHROMagar, 2021. CHROMagar™ ECC – For the simultaneous detection of *E. coli* and coliforms in water and food samples. Available at: https://www.chromagar.com (accessed on 2015)

Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R. and Rouphael, Y., 2015. Protein hydrolysates as biostimulants in horticulture. *Scientia Horticulturae*, 196, 28–38. DOI:10.1016/j.scienta.2015.08.037

Dong, P., Xiong, F., Que, Y., Wang, K., Yu, L. and Li, Z., 2017. Expression profiling and functional analysis reveals that TOR is a key player in regulating photosynthesis and phytohormone signaling in Arabidopsis. *Frontiers in Plant Science*, 8, 63. DOI:10.3389/fpls.2015.00677

Esakkiraj, P., Meleppat, B., Lakra, A. K., Ayyanna, R. and Arul, V., 2016. *Cloning, expression, characterization and application of protease produced by Bacillus cereus PMW8*. RSC Advances, **6**, 38611–38616. DOI: 10.1039/C5RA27671C

Forde, B.G. and Lea, P.J., 2007. Glutamate in plants: metabolism, regulation, and signalling. *Journal of Experimental Botany*, 58, 2339–2358. DOI:10.1093/jxb/erm121

Gaskell, M. and Smith, R., 2007. Nitrogen sources for organic vegetable crops. *Hort Technology*, 17, 431–441. DOI:10.21273/HORTTECH.17.4.431

Heidarzadeh, A., 2025. Role of amino acids in plant growth, development, and stress responses: a comprehensive review. *Discover Plants*, 2, 1–32. DOI:10.1007/s44372-025-00322-0

Henderson, J.W., Ricker, R.D., Bidlingmeyer, B.A. and Woodward, C., 2000. Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids. Agilent Technologies Technical Note, 5980-1193.

Illera-Vives, M., Seoane Labandeira, S. and López-Mosquera, M.E., 2013. Production of compost from marine waste: evaluation of the product for use in ecological agriculture. *Journal of Applied Phycology*, 25, 1395–1403. DOI:10.1007/s10821

ISO, 1995. ISO 10694:1995 - Soil quality — Determination of organic and total

carbon after dry combustion (elementary analysis). Geneva, Switzerland.

ISO, 1998. ISO 15214:1998 - Microbiology of food and animal feeding stuffs — Enumeration of mesophilic lactic acid bacteria — Colony-count technique at 30°C. Geneva, Switzerland.

ISO., 2005. ISO 13903:2005 - Animal feeding stuffs — Determination of amino acids content. Geneva, Switzerland.

ISO., 2008. ISO 21527-1:2008 - Enumeration of yeasts and moulds — Part 1: Colony count in high water activity products. Geneva, Switzerland.

ISO, 2013. ISO 4833-1:2013 - Enumeration of microorganisms — Colony count at 30°C by pour plate technique. Geneva, Switzerland.

ISO, 2014. ISO 9308-1:2014 - Water quality — Enumeration of E. coli and coliform bacteria — Membrane filtration method. Geneva, Switzerland.

Karim, N.U., Lee, M.F.M.A. and Arshad, A.M., 2015. The effectiveness of fish silage as organic fertilizer on post-harvest quality of pak choy (*Brassica rapa* L. subsp. chinensis). *European International Journal of Science and Technology*, 4, 163–174. DOI:10.53797/agrotech.v1i1.6.2022

Lal, R., 2020. Soil organic matter content and crop yield. *Journal of Soil and Water Conservation*, 75, 123A–124A. DOI:10.2489/jswc.75.2.27A

Lee, S., Ahn, S. and Zong, J., 2025. Sustainable valorization of fish head wastes into nitrogen based nutrients: a comparative life cycle assessment. *Journal of Biological Engineering*, 19, 87- 98 DOI:/10.1186/s13036-025-00560-6

Lee, S.H., Kang, D.H. and Lee, Y.H., 2016. Lactic acid bacteria in fish waste silage and their impact on fermentation. *Food Science and Biotechnology*, 25, 1571–1578. DOI:10.3390/ani1508115.

Liu, H., Chen, D. and Zhang, R., 2016. Amino Acids Hydrolyzed from Animal Carcasses Are a Good Additive for the Production of Bio-organic Fertilizer. *Frontiers in Microbiology,* 7, 1–11. DOI:10.3389/fmicb.2016.01290

Madende, M. and Hayes, M., 2020. Fish By-Product Use as Biostimulants: An Overview of the Current State of the Art, Including Relevant Legislation and Regulations within the EU and USA. *Molecules*, 25, 11-22. DOI:10.3390/molecules25051122

Mahdavi, Z., Esmailpour, B., Azarmi, R., Panahirad, S., Ntatsi, G., Gohari, G., and Fotopoulos, V., 2024. Fish Waste — A Novel Bio-Fertilizer for Stevia (Stevia rebaudiana Bertoni) under Salinity-Induced Stress. *Plants*, 13, 1-19. DOI:10.3390/plants13141909

Marschner, P., 2012. Marschner's Mineral Nutrition of Higher Plants, third ed. Academic Press, Elsevier publication, 649 P.

Marti-Quijal, F.J., Remize, F., Meca, G., Ferrer, E., Ruiz, M.J. and Barba, F.J., 2020. Fermentation in fish and by-products processing: An overview of current research and future prospects. *Current Opinion in Food Science*, 31, 9–16. DOI:10.1016/j.cofs.2019.08.001

Mehta, N.K., Sharma, S., Triphati, H.H., Satvik, K., Aruna, K., Choudhary, B.K. and Meena, D.K., 2023. A waste

to wealth strategies for greening of the environment commodities. Conversion of fish processing waste to value-added. *In Advances in Resting-state Functional MRI*, Woodhead Publishing, pp. 421–466. DOI:10.1016/B978-0-323-99145-2.00005-7

Moore, S., 2004. Amino acid analysis: Methods and protocols. *Methods in Molecular Biology*, 243, 39–57. DOI:10.1385/1-59259-644-6:039

Nuzhyna, N., Raksha, N., Savchuk, O., Maievska, T. and Tonkha, O., 2025. Hydrolysates of fish waste as potential plant biostimulants. *International Journal of Recycling of Organic Waste in Agriculture*, 14, 22-35. DOI:10.57647/ijrowa.2025.16870

Peñarubia, O.R., Toppe, J. and James, D., 2020. Fish waste management: Turning waste into healthy feed with antimicrobial properties. *Asian Fisheries Science*, 33, 11–15. DOI:10.33997/j.afs.2020.33.S1.002

Radziemska, M., Vaverková, M.D., Adamcová, D., Brtnický, M. and Mazur, Z., 2019. Valorization of fish waste compost as a fertilizer for agricultural use. *Waste and Biomass Valorization*, 10, 2537–2545. DOI:10.1007/s12649-018-0288-8

Raeesi, R., Shabanpour, B. and Pourashouri, P., 2023. Use of fish waste to silage preparation and its application in animal nutrition. *Journal of Animal and Feed Research*, 13, 79–88. DOI:10.51227/ojafr.2023.13

Rustad, T., Storrø, I. and Slizyte, R., 2011. Possibilities for the utilisation of marine by-products. *International Journal of Food Science and Technology*, 46, 2001–2014. DOI:10.1111/j.1365-2621.2011.02736.x

Safari, R., Nasrollahzadeh, H., Farabi, M.V. and Jafari, A., 2020. Production of Biological Silage from Poultry Waste and Its Impact on Growth Indices and Immunity of Rainbow Trout. Management and Planning Organization of Mazandaran Province. Iran. 117 P.

Safari, R., Nasrollahzadeh, H., Farabi, M.V. and Jafari, A., 2021. Production of Biological Silage from Poultry Waste and Its Impact on Growth Indices and Immunity of Rainbow Trout. Management and Planning Organization of Mazandaran Province, Iran. pp 112–117.

Shahidi, F. and Synowiecki, J., 1997. Protein hydrolysates from seal meat as phosphate alternatives in meat products. *Food Chemistry*, 60, 29–34. DOI:10.1016/S0308-8146

Sarhadi, N., Motamedzadegan, A., Taheri, A. and Azad, M., 2012. Comparative study of nutrient composition and amino acid profile in bones of Golden Side Sardine (*Sardinella gibossa*), Anchovy Kilka (*Clupeonella engrauliformis*), and Indian Mackerel (*Stolephorus indicus*). *Iranian Scientific Fisheries Journal*, 21, 101–112. DOI:10.22092/isfj.2017.110042

Shenbagavalli, S., Prabhu, T. and Ponmani, S., 2020. Development of fish waste compost and its effect on quality of soil and crop. *International Journal of Chemical Studies*, 8, 484–488.

Shobihah, H. N., Andriani, Y. and Wiyatna, M. F., 2025. A review on

optimizing fish growth using fermented animal by-products for sustainable aquaculture. *Journal of USK*, 14, 226–235. DOI:10.13170/depik.14.2.43418

Iranian Fisheries Statistical Yearbook, 2024. Statistical Yearbook of the Iranian Fisheries Organization. Planning and Budget Office, Planning and Resource Management Deputy, Iranian Fisheries Organization, Iran. 64 P.

USEPA, 2007. Method 7000B: Flame Atomic Absorption Spectrophotometry. U.S. Environmental Protection Agency, Washington, D.C.

Wang, Y., Li, Q. and Li, C., 2023. Organic fertilizer has a greater effect on soil microbial community structure and carbon and nitrogen mineralization than planting pattern in rainfed farmland of the Loess Plateau. *Frontiers in Environmental Science,* 11, 1–12. DOI:10.3389/fenvs.2023.1232527

Wang, H., Cui, Z. and Gao, Y., 2025. Advances on microbial mechanisms of nitrogen transformation during nitrification and denitrification at soil aggregates scale. *Applied Soil Ecology*, 213, 311–326. DOI:10.1016/j.apsoil.2025.106326

White, P.J. and Broadley, M.R., 2003. Calcium in plants. *Annals of Botany*, 92, 487–511. DOI:10.1093/aob/mcg164

Woo, P.C., Lau, S.K., Teng, J.L., Tse, H. and Yuen, K.Y., 2008. Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clinical Microbiology and Infection*, 14, 908–914. DOI:10.1111/j.1469-0691.2008.02070.x

Xu, G., Fan, X. and Miller, A.J., 2021. Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, 72, 233–260. DOI:10.1146/annurev-arplant-042811-105532

Zhang, C., Zhang, R., Gao, X. and Wang, L., 2017. Preparation of amino acid liquid fertilizer from fish waste hydrolysate and its application on plant growth. *Journal of Cleaner Production*, 156, 610–618. https://doi.org/10.1016/j.jclepro.2017.04.095

Zhang, H., Li, H. and Zhou, Y., 2018. Optimization of enzymatic hydrolysis of fish waste and amino acid characteristics. *Food Chemistry*, 240, 965–972. DOI:10.15578/squalen.v9i3.79