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Agricultural Bio-Stimulant Activity of Fish Protein Hydrolysate from Common Silver Belly (*Gerres subfasciatus*)

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Abstract

Taking into account of using the fish industry wastes and for expanding the incentive to a few under used fish species, protein hydrolysates from fish proteins are being set up by a few specialists everywhere throughout the world. Fish Protein Hydrolysates (FPH) are breakdown results of enzymatic transformation of fish proteins into smaller units like peptides, which typically contain 2-20 amino acids. Their worth has been expanded by utilizing them as fluid bio compost, feed supplement and bioorganic fertilizer. From the research carried out at our laboratory, Dry fish silver belly with both microbe and enzyme acquired from cultural broth of lactobacillus species, catalyst papain demonstrated positive upgrade of development and advancement of plants tested. In view of the yield of FPH concentrates from both microbial and enzymatic hydrolysis, the pace of seedling life list in Urad dhal, green gram and okra got higher in 0.1-0.5% convergence of dry fish hydrolysate from lactobacillus treated than in the control variation. With respect to the seeds of urad dhal, green gram and okra, the germination rate and seedling energy list from treated seeds surpassed control by 19.11-128.71%. The positive effect of dry fish protein hydrolysate by microbial procedure on germination rate, seedling life record rate in 3 kinds of seeds is the reason for the improvement of protein hydrolysate item for application in crop creation utilizing dry fish squanders. The Seedling grown in 0.5% concentration of fish hydrolysates showed significant increase in seedling vigour index of Urad dhal (13.29 ± 0.12 %), Green gram (14.1 ± 0.17%) and Okra (5.39 ± 0.43 %) was recorded through one way Analysis of Variance (ANOVA). By HPLC determination amino acid results determined that protein hydrolysates process can be stopped on 7th day (643.56ppm) whereas @ 10th day amino acid decreased (326.24ppm). Results suggest that probiotic assumes a superior job in dry fish protein hydrolysates than compounds when contrasted and yield and prudent astute.

Keywords: Silver belly; Enzymatic hydrolysis; Protein hydrolysates; Amino acid profile; Agro bio-stimulant

Introduction

Every year around 91 million tons of fish are collected of which fish dinner about 29.5% is changed [1,2]. Over half of the rest of the fish tissue viewed as waste and not taken as nourishment [3]. Traditionally fish squander has additionally utilized as a manure creating nutritive components (N and P) and their speedy decay. Numerous activities utilizing fish squander came about mostly from aquaculture have turned out in various pieces of the world in looking of inconsequent and significant procedures for changing over fish squander into helpful farming items [4-6]. Fish waste from farm upsets the region in and around legitimately brought about by the effluent. Yet in addition changes an expansive waterfront zone at different biological system levels, along these lines diminishing the biomass, tiny fish and nekton [7,8] thickness and assorted variety of benthos, adjusting characteristic nourishment networks.

As per the report in 2009, fish contributed about 16.6% of the world's complete admission of creature protein yet just 6.5% of all the protein exists on the planet [9]. Practically the majority of the fish wastes are arranged in the sea. Natural issue was separated by the high-impact microbes present in the water prompting a gigantic decrease of oxygen in water. Different in pH by over-burdens of N, NH_3 and P raised turbidity of water and because of this alga, deterioration happens. Because of decrease in water, oxygen content created anaerobic condition which prompts the arrival of H_2S and NH_3, natural corrosive, CO_2, CH_4 [10].

The majority of the fish contains 15-30% of protein, 0-20% of fat substance and Moisture 50-80% [11]. Amino corrosive arrangement was even in fish protein. 16-18 amino corrosive in fish rely on the species and regular varieties [12,13]. Because of high amino corrosive substance in fish, it has been utilized as fish sauce, fish supper, creature feed, fish silage and manure [14]. Roughly, the loss from fish handling plant recorded to 20 million tons that is equivalent to 25% of the world's absolute creation from fisheries catching region [15]. For instance nutritive estimation of silver paunch fish involved Moisture-91g; fat-19g; Mineral-2g; fiber-3g; starch-0g; Calcium-715mg;

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phosphorous-741mg; iron-2mg [16]. FPH treatment at 2ml/L had also been shown to increase seed vigour, Guaiacol Peroxidase (GuPX) activity, Glucose-6-Phosphate Dehydrogenase (G6PDH) activity and phenolic content in pea during its earlier stages of germination [17].

The past research contemplates investigated the enzymatic handling of fish protein is by all accounts lab or little scope oriental however there is limitation when scaled up to huge scope [18]. Because of the nearness of fundamental supplements and bioactive segments in fish protein hydrolysates, these find place in different modern applications. Protein hydrolysates are breakdown results of enzymatic change of proteins into littler peptides. These protein hydrolysates are delivered by the enzymatic hydrolysis of local proteins [19]. Laboratory has been researching the impact of Fish Protein Hydrolysates (FPH) on seedling power [20]. Nations like Japan, France and a few nations like South-East Asia were done enormous scope creation of fish protein hydrolysate. In any case, the procedure has a few weaknesses like essential significant expense of catalysts, low yields, and chemical inactivation after hydrolysis by pH or by heat lastly the powerlessness to reuse proteins [21]. Extraction of protein by compounds is completed under settled pH condition without upsetting their wholesome characteristics for the acknowledgment in the enterprises like nourishment, and wide range of items can be produced for an expansive scope of uses [22].

Use of protein hydrolysates in a wide assortment of employments in ventures like nourishment, protein supplements, enhance enhancers, drink as stabilizers and milk replacers industry. Business fish waste or dry fish were utilized to deliver fish silage by microbial processing under anaerobic maturation estimated 50g or 50ml/kg LB culture and 150g or 150ml/kg sugarcane molasses [23]. The basic Silver stomach is types of significant which is the local spot of Pacific and Indian seaside waters of Australia. Its morphological structure is Silver shaded body, which can grow up to 20cm long. It circulates from Southwestern Australia in the area of the tropical north of Australia and in the south on the east coast to Southern New South Wales.

The target of this investigation is to decide the ideal conditions for the protein hydrolysate creation procedure of dry fish protein by compound and probiotic culture. Just as investigation intended to assess the amino corrosive structure of fish protein hydrolysate delivered under different fixation levels in a period subordinate way. In the meantime, the preliminary utilizing Probiotic culture proceeded for additional 10 days to watch the amino corrosive profile content in the up and coming days (third day, seventh day and tenth day). Amino corrosive profile content was totally concentrated by HPLC 1260 Infinity II in crude material, Quick wash, third day, seventh day, tenth day and tenth day of the buildup).

Materials and Methods

For the whole experimental study, specifically the common silver belly species of dry fish were collected from the place Mandapam (Tamilnadu) Coastal region. Collected dry fish moved to AquAgri Processing Pvt. Ltd, Manamadurai, Tamilnadu to carry out further process. Both enzymes and probiotic based processes carried out in common silver belly dry fish.

Sample preparation

The materials were rinsed with water in the ratio of 1:2 to remove the impurities and other foreign materials. Washed dry fish were chopped into small pieces before homogenized.

Preparation of fish protein hydrolysates (FPH): The planning of Dry fish protein hydrolysate was led by the technique for klompong et al. with the change. The dry fish and water (1:3) (W/W) were utilized for the homogenization procedure. The minced dry fish treated with different centralizations of compounds at different temperatures in time subordinate way. After treatment, the filtrate sifted through muslin material. In protein process, the filtrate was warmed at 90°C for 20min so as to inactivate the endogenous chemical and mixed constantly utilizing an attractive stirrer. Remaining buildup dried under daylight and grounded. The Same washed dry fish was treated with probiotic culture with different centralizations of the substrate (Jaggery) for 72hrs. In the interim dry fish without catalyst and probiotic culture were treated as control.

Enzymatic hydrolysis: Papain catalyst (B.No: VS/PAP 01/001) is bought from V Sthiraa Bioscience E-101, Jay Ambe Residency, B/H. Anand Vatika, Mota Varachha, Dist-Surat, Gujarat (India). Enzymatic hydrolysis completed at different convergences of papain proteins under time subordinate way and different temperatures based. Different Concentrations of catalyst (2%, 4% and 6%) followed for the investigation with different temperatures (40-45°C, 60-65°C, 75-80°C). Treatment completed at a time interim of 5 hours, 10 hours and 15 hours.

Microbial digestion of dry fish: Lactic acid bacteria used in dry fish protein hydrolysis with various concentrations of Jaggery substrate. Lactic acid bacteria were isolated through rice wash water method and the isolated culture was enriched with 30% of Jaggery to keep virulent for further use. Isolated colonies on MRS agar gram stained and biochemical tests carried out to confirm the species level.

Physico-chemical parameter analysis

Moisture content: Moisture content of the sample was determined by placing approximately 2g of sample into a pre weighed petri dish. Samples kept in an oven and dried at 105°C for 4-5 hours until constant weight obtained [24].

Quantification of Amino acid through HPLC: Acetonitrile (LC grade), methanol (LC grade) and LC grade Millipore water were purchased. Borate buffer, OPA and FMOC reagents and standard solutions of mixture of 17 amino acids (25, 100, 250 and 1000 nmol cm^{-3}) were obtained from Agilent Technologies (Waldbronn, Germany). Hydrochloric acid, used for the preparation of 6mol dm^{-3} and 0.1mol dm^{-3} HCl, was obtained Cellulose membrane syringe filter (0.22µm pore size), screw cap vials and screw caps were purchased from Agilent Technologies (Waldbronn, Germany). The analysis was performed on an Agilent 1260 Infinity l l liquid chromatography system, equipped with a 1260 Quaternary pump (G7111B), 1260 standard auto sampler (G1729A), 1260 thermo stated column compartment, 1260 diode array and multiple wavelength detector (G7115A), and a Poroshell 120 EC-C18 column (100mm × 4.6mm, i.e., particle size 2.7µm) (Agilent Technologies). Fish hydrolysate was analyzed for their amino acid content. The solutions were filtered through quantitative filter paper into glass tubes and the filtrates were

Table 1: Detail of the trial code and their treatment.

Treatment	Trial	Temp (°C)	Enzyme conc. (w/w)	Initial Weight (gm)	Fish: Water Ratio	Time (min)
Papain Enzyme	T1	40-45	2	100	1:02	5
	T2	40-45	2	100	1:02	10
	T3	40-45	2	100	1:02	15
	T4	40-45	4	100	1:02	5
	T5	40-45	4	100	1:02	10
	T6	40-45	4	100	1:02	15
	T7	40-45	6	100	1:02	5
	T8	40-45	6	100	1:02	10
	T9	40-45	6	100	1:02	15
	T10	60-65	2	100	1:02	5
	T11	60-65	2	100	1:02	10
	T12	60-65	2	100	1:02	15
	T13	60-65	4	100	1:02	5
	T14	60-65	4	100	1:02	10
	T15	60-65	4	100	1:02	15
	T16	60-65	6	100	1:02	5
	T17	60-65	6	100	1:02	10
	T18	60-65	6	100	1:02	15
	T19	75-80	2	100	1:02	5
	T20	75-80	2	100	1:02	10
	T21	75-80	2	100	1:02	15
	T22	75-80	4	100	1:02	5
	T23	75-80	4	100	1:02	10
	T24	75-80	4	100	1:02	15
	T25	75-80	6	100	1:02	5
	T26	75-80	6	100	1:02	10
	T27	75-80	6	100	1:02	15
	T28	RT	Control	100	1:02	5
	T29	RT	2	100	1:02	5
	T30	RT	4	100	1:02	5
	T31	RT	6	100	1:02	5
	T32	RT	Control	100	1:02	10
	T33	RT	2	100	1:02	10
	T34	RT	4	100	1:02	10
	T35	RT	6	100	1:02	10
	T36	RT	Control	100	1:02	15
	T37	RT	2	100	1:02	15
	T38	RT	4	100	1:02	15
	T39	RT	6	100	1:02	15
Probiotic (LB)	T40	RT	5% Jaggery	100	1:02	72hrs
	T41	RT	10% Jaggery	100	1:02	72hrs
	T42	RT	20% Jaggery	100	1:02	72hrs
	T43	RT	30% Jaggery	100	1:02	72hrs

purified using 0.22µm pore size, cellulose membrane syringe filter [21].

HPLC Determination: The chromatographic conditions employed were in accordance with the Agilent method [22]. The mobile phase A consisted of 5.678g of Na_2HPO_4 + 15.2g of $Na_2B_4O_7.10H_20$) per 4 liters of water, adjusted to the pH 8.2 with Conc. HCl solution. The mobile phase B was acetonitrile-methanol-water (45:45:10, vol. %). Briefly, the hydrolyzed samples or the solutions the standard amino acid mixture were automatically derivatised with OPA and FMOC by programming the autosampler (1. draw 2.5µl from vial 1 (borate buffer); 2. draw 0.5µl from sample (position X); 3. mix 3µl in air, max. speed, 2×; 4. wait 0.5min; 5. draw 0µl from vial 2 (water, uncapped vial); 6. draw 0.5µl from vial 3 (OPA); 7. mix 3.5µl in air, max speed, 6×; 8. draw 0µl from vial 2 (water, uncapped vial); 9. draw 0.5µl from vial 4 (FMOC); 10. mix 4µl in air, max speed, 6×; 11. draw 32µl from vial 5 (water); 12. mix 18µl in air, max speed, 2× and 13. inject). After derivatisation, 0.5µl of each sample was injected into a Poroshell column at 40°C, with detection at λ1 = 338nm and λ2 = 262nm.

Sample Preparation for HPLC analysis: Ten grams of solid material of dry fish and 40ml of extracting solvent (75% methanol in distilled deionized water) were added to a Mason jar and blended for 2min. The homogenate was then transferred to a 100ml volumetric flask and then the jar rinsed three times with water, which was then brought up to volume and stored overnight at 4°C. The contents of the flask were transferred to a 50ml centrifuge tube and centrifuged at 15000rpm for 40min. The supernatant was filtered using Nylon 0.2µm filter membrane and treated as per standard solution. The liquid extract was transferred to a 50ml centrifuge tube and centrifuged at 5000rpm for 30min. The supernatant was filtered through Nylon 0.2µm filter membrane and treated as per standard solution [25].

Efficacy study

To consider the adequacy of LB processed protein hydrolysate, germination study completed by seed treatment process. Roughly, 10 seeds treated with different groupings of dry fish protein hydrolysate (0.1, 0.5, 1.0, 1.5, and 2.0%). Randomly selected seeds like Urad dhal, Green gram, Okra were absorbed referenced above fixations for 60 minutes. The treated seeds were placed in the germination sheet for observation.

Statistical analysis

Statistical analysis of the data was performed by one way ANOVA. Data sets with P values <0.05 or <0.1 were considered statistically significant. The experiment was repeated for five times and the data sets combined for analysis.

Results

Total solids of fish hydrolysate calculated at various concentrations in a time dependent manner and temperature. According to time dependent manner, enzyme concentration of 2% at 60-65°C for 5hrs have shown the highest TS value when compared with other time duration (10hrs & 15hrs). Reduction in total solid at higher temperature in all concentration of enzyme is illustrated in Table 3. Results obtained for 3 different control under 3 different time duration (5, 10 and 15 hrs) have shown that the gradual increase

Table 2: Total Solid content of the various trials.

Treatment	Trial	Temp (°C)	Enzyme conc. (w/w)	Recovered Volume	TS%	Recoverable Solid/100gm	pH
Papain Enzyme	T1	40-45	2	110	28.43	31.27	5.66
	T2	40-45	2	140	25.47	35.66	5.62
	T3	40-45	2	68	39.39	26.78	5.67
	T4	40-45	4	112	30.91	34.62	5.62
	T5	40-45	4	130	30.55	39.71	5.68
	T6	40-45	4	70	40.83	28.58	5.67
	T7	40-45	6	136	32.88	44.72	5.58
	T8	40-45	6	140	32.62	45.67	5.63
	T9	40-45	6	72	45.44	32.72	5.68
	T10	60-65	2	86	37.57	32.31	5.59
	T11	60-65	2	130	30.85	40.1	5.64
	T12	60-65	2	132	29.93	39.51	5.66
	T13	60-65	4	106	38.32	40.62	5.6
	T14	60-65	4	110	35.03	38.53	5.59
	T15	60-65	4	134	34.82	46.66	5.64
	T16	60-65	6	112	38.63	43.26	5.58
	T17	60-65	6	170	29.69	50.47	5.62
	T18	60-65	6	158	30.84	48.72	5.67
	T19	75-80	2	200	19.8	39.6	5.65
	T20	75-80	2	140	28.7	40.18	5.63
	T21	75-80	2	210	23.2	48.72	5.67
	T22	75-80	4	200	22.6	45.2	5.41
	T23	75-80	4	150	29.7	44.55	5.58
	T24	75-80	4	230	20.2	46.46	5.62
	T25	75-80	6	200	24.2	48.4	5.65
	T26	75-80	6	170	27.6	46.92	5.64
	T27	75-80	6	200	24.1	48.2	5.58
	T28	RT	Control	105	15.9	16.69	5.98
	T29	RT	2	135	19.4	26.19	5.72
	T30	RT	4	145	20.6	29.87	5.74
	T31	RT	6	145	24.2	35.09	5.75
	T32	RT	Control	115	19.6	22.54	5.97
	T33	RT	2	150	21.8	32.7	5.73
	T34	RT	4	158	22.6	35.71	5.74
	T35	RT	6	160	25.6	40.96	5.76
	T36	RT	Control	118	21.2	25.02	5.99
	T37	RT	2	152	22.9	34.81	5.73
	T38	RT	4	160	24.2	38.72	5.76
	T39	RT	6	165	26.8	44.22	5.74
Probiotic (LB)	T40	RT	5% jiggery	150	20.65	30.97	6.75
	T41	RT	10% jiggery	160	23.18	37.09	6.11
	T42	RT	20% jiggery	165	26.02	42.93	4.35
	T43	RT	30% jiggery	170	27.84	47.33	4.31

in TS from 5 to 15 hrs with 15.9%, 19.6% and 21.2% respectively. But on treated (60-65°C at 2% for 5hrs) have shown the higher TS value (37.57%). In probiotic culture with various concentration of substrate Jaggery (5%, 10%, 20% and 30%) have shown highest TS value of 27.84% after 3 days with pH-4.31 in 30% substrate Jaggery added. Control (without adding of probiotic culture with a substrate Jaggery) was quickly contaminated within 24hrs.

Influence of DFH on germination

The germination percentages, average seedling height and seedling vigour index of Urad dal, Green gram and Okra seeds following the DFH treatment are shown in the Table 3. Development of root length and shoot length in treated seeds compared with control. In treated urad dal seed 100% of seed germination rate at maximum number of concentration (0.1, 0.5, 1.0 and 1.5%) whereas in control 86 ± 5.48% of seed germination was observed. As expected, 80 ± 7.07% of seed germination occurred in highest concentration (2%), it meant that it has shown the suppressing activity of seed. It was shown in Figure 1. All seedlings appeared to respond positively at lowest concentration (0.5%) of DFH. Similarly, in green gram higher SVI (14.14 ± 0.17%) in 0.5% concentration, which is significantly correlated with control in which it was only 6.66 ± 0.55%, was obtained and shown in Figure 2. In okra seed 76 ± 5.48% and 96 ± 5.48% of the seed germination was obtained in control and 0.5% respectively. SVI have shown the highest rate of 5.39 ± 0.43% at 0.5%, control with 2.80 ± 0.28% and 1.91 ± 0.47% at 2%, which was observed in Figure 3.

Amino acid profile content

Amino analysis report was predicted for 8 number of samples include commercial product I (Amino acid rich Sap), commercial product II (Fish hydrolysate), papain enzyme treated dry fish hydrolysate liquid & residue and Probiotic treated dry fish hydrolysate liquid & residue. High protein content was observed in Probiotic Lactobacillus residue when compared with other samples. Total Solid (42.53%) and Protein content (24.98%) was rich in commercial product II liquid sample whereas papain enzyme treated liquid sample have shown TS - 15.96% and Protein - 11.27%. Alike Probiotic treated liquid sample have shown TS - 18.29% and Protein - 5.64%. Both the residues of papain enzyme treated (Protein content - 48.57%) and Probiotic treated residue (Protein content - 57.33%) have shown the highest protein content compared with other liquid hydrolysate sample. Amino acid profiles of both enzymatic hydrolysis and Probiotic hydrolysis of dry fish are individually recorded in the Table

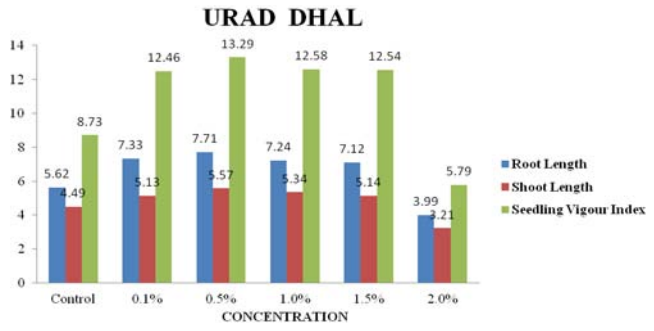

Figure 1: Different Concentration of Fish Hydrolysate on germination of Urad dhal.

Table 3: Germination percentage by treatment.

Seed	Concentration of DFH	Root Length	Shoot Length	Plant Length	Germination Percentage	Seedling Vigour Index
Urad Dal	Control	5.62 ± 0.64	4.49 ± 0.31	10.11 ± 0.92	86 ± 5.48	8.73 ± 1.28
	0.1	7.33 ± 0.39	5.13 ± 0.24	12.46 ± 0.60	100 ± 0	12.46 ± 0.60
	0.5	7.71 ± 0.09	5.57 ± 0.11	13.29 ± 0.14	100 ± 0	13.29 ± 0.14
	1	7.24 ± 0.12	5.34 ± 0.05	12.58 ± 0.10	100 ± 0	12.58 ± 0.10
	1.5	7.12 ± 0.14	5.14 ± 0.08	12.25 ± 0.19	100 ± 0	12.54 ± 0.19
	2	3.99 ± 0.38	3.21 ± 0.23	7.19 ± 0.61	80 ± 7.07	5.79 ± 0.96
Green Gram	Control	5.25 ± 0.08	3.52 ± 0.14	8.76 ± 0.14	76 ± 5.48	6.66 ± 0.55
	0.1	8.15 ± 0.04	5.31 ± 0.09	13.46 ± 0.09	100 ± 0	13.46 ± 0.09
	0.5	8.61 ± 0.07	5.54 ± 0.11	14.14 ± 0.17	100 ± 0	14.14 ± 0.17
	1	8.32 ± 0.02	4.73 ± 0.02	13.04 ± 0.03	100 ± 0	13.04 ± 0.03
	1.5	6.74 ± 0.15	3.94 ± 0.02	10.67 ± 0.16	86 ± 8.94	9.19 ± 1.06
	2	5.36 ± 0.05	3.24 ± 0.09	8.71 ± 0.11	80 ± 10	6.89 ± 0.91
Okra	Control	2.48 ± 0.1	1.21 ± 0.03	8.76 ± 0.14	76 ± 5.48	2.80 ± 0.28
	0.1	3.58 ± 0.04	2.11 ± 0.06	13.46 ± 0.09	94 ± 5.48	5.35 ± 0.35
	0.5	3.59 ± 0.07	2.02 ± 0.01	14.14 ± 0.17	96 ± 5.48	5.39 ± 0.43
	1	2.79 ± 0.13	1.79 ± 0.15	13.04 ± 0.03	86 ± 5.48	3.94 ± 0.47
	1.5	2.18 ± 0.11	1.02 ± 0.05	10.67 ± 0.16	74 ± 5.48	2.37 ± 0.24
	2	1.91 ± 0.04	0.66 ± 0.23	8.71 ± 10.94	62 ± 4.47	1.91 ± 0.47

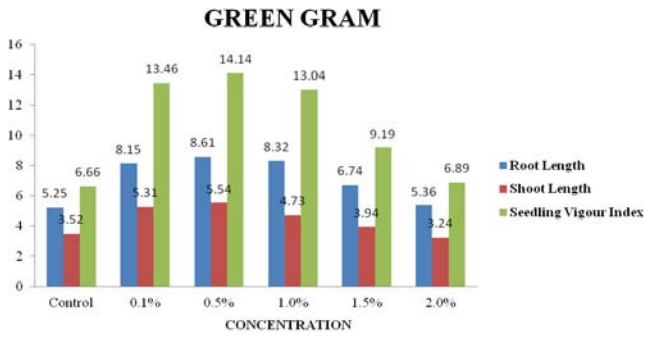

Figure 2: Different Concentration of Fish Hydrolysate on germination of Green gram.

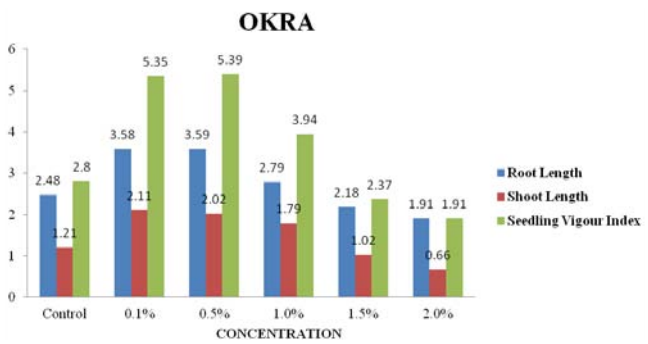

Figure 3: Different Concentration of Fish Hydrolysate on Okra.

4. Amino acid profile of fish hydrolysate extracts (Probiotic culture) is illustrated in Table 5. The overall response to DFH treatment by each extract was different. When comparing 3rd and 7th day of DFH almost all the amino acid content is similar, only 2-5 amino acids increased on 7th day when compared with 3rd day. But on the other hand, amino acid content of DFH decreased on 10th day.

Discussion

Liquid produces obtained from fish by proteolytic enzymes or probiotics under various conditions of digestion results in fish protein hydrolysates. Requirement of alternative protein source has increased all around world over last decade. High quality proteins are available in enormous amounts from fish, fish protein hydrolysate and their value of importance has been increased by using them as a food supplement, bio-organic manure and liquid bio fertilizer. As a typical side effect of the fisheries business, FPH is known to be wealthy in amino acids basic to Proline metabolism [3]. Numerous investigations detailed the organic action of different fish protein hydrolysates in vivo and in vitro by methods for their bioactive peptides [26-29]. Scientists explained the protein substance of fish protein hydrolysates went between 60% to 90% of complete arrangement [30-38]. Fish protein hydrolysates have been accounted for to show variation in their amino corrosive organization [34,39,40]. As a run of the mill reaction of the fisheries business, FPH is known to be well off in amino acids fundamental to proline metabolism [3]. Various examinations point by point the normal development of various fish protein hydrolysates *in vivo* and *in vitro* by strategies for their bioactive peptides [26-29].

The variety in amino corrosive creation of various fish protein hydrolysates for the most part relies upon a few factors, for example, crude material, catalyst source, and hydrolysis conditions [41,42]. Fish protein hydrolysate production using bacterial fermentation is better than all other mode of actions. Bacterial fermentation initiated by adding sugar or molasses as a substrate which helps to grow lactic acid, antibiotics which combine eliminate spoilage bacteria and

Table 4: Typical analysis report (Hydrolysate and Residue) for digested dry fish using papain enzyme and probiotic.

S. No.	Parameters	Commercial Product-1	Commercial Product-2	Protein hydrolysate (Papain Enzyme)	Papain Enzyme - Residue	Protein hydrolysate (Probiotic LB)	Probiotic LB - Residue
1	Total Solids	12.99	42.53	15.96		18.29	
2	Protein	6.78	24.98	11.27	48.57	5.64	57.33
3	Total Carbohydrate					6.42	11.73
4	Fat					0.39	4.73
5	Aspartic + ASN	0	0.56	0.17	3.62	0.22	2.98
6	Serine	0.04	0.25	0.03	1.6	0.14	1.66
7	Glutamic + GLN	0.84	0.67	1.6	5.71	0.35	4.49
8	Glycine	0.38	0.76	0.14	3.82	0.37	2.86
9	Histidine	0	0.15	0.03	0.89	0.06	0.85
10	Arginine	0.27	0.27	0.02	2.67	0.4	2.98
11	Threonine	0	0.23	0.04	1.55	0.13	1.72
12	Alanine	0.1	0.55	0.15	2.65	0.26	2.3
13	Proline	1.53	0.54	0.31	2.31	0.25	1.97
14	Cystine	2.76	0	0	0	0	0
15	Tyrosine	0	0.23	0.06	1.52	0.09	1.59
16	Valine	0.07	0.36	0.1	2.22	0.17	2.15
17	Methionine	0.05	0.01	0.1	0.69	0	0.76
18	Lysine	0.01	0.44	0.08	2.94	0.21	3.11
19	Isoleucine	0	0.3	0.05	1.95	0.12	1.91
20	Leucine	0.01	0.46	0.09	3	0.21	3.24
21	Phenylalanine	0	0.23	0.04	1.82	0.11	1.93
22	Tryptophan	0	0.02	0.04	0.44	0.01	0.5
		6.06	6.03	3.05	39.4	3.1	37
23	Free Aspartic + ASN	0	0.04	0.01	0.09	0.18	0.05
24	Free Serine	0	0.03	0	0.06	0.04	0.02
25	Free Glutamic + GLN	0.52	0.06	1.07	0.2	0.23	0.14
26	Free Glycine	0.23	0.1	0	0.12	0.1	0.12
27	Free Histidine	0	0	0	0	0	0.11
28	Free Arginine	0.1	0.05	0	0.1	0.04	0.12
29	Free Threonine	0	0.03	0	0.08	0.05	0.05
30	Free Alanine	0.01	0.05	0.01	0.16	0.19	0.22
31	Free Proline	0.79	0.11	0.19	0.08	0.08	0.1
32	Free Cystine	0.28	0.06	0	0.08	0.11	0.06
33	Free Tyrosine	0	0.04	0.01	0.12	0.08	0.05
34	Free Valine	0	0.08	0.03	0.15	0.08	0.13
35	Free Methionine	0.12	0.04	0.01	0.04	0.25	0.2
36	Free Lysine	0.06	0.03	0.03	0.02	0.17	0.12
37	Free Isoleucine	0	0.04	0	0.08	0.2	0.15
38	Free Leucine	0.01	0.15	0	0.28	0.17	0.14
39	Free Phenylalanine	0	0.06	0	0.11	0.21	0.26
40	Free Tryptophan	0	0	0.06	0.03	0.07	0.14
	Total	2.12	0.97	1.42	1.796	2.25	2.18

Table 5: Amino acid profile content of DFH on different time periods of extract.

Amino Acid Profile	QW	3rd Day	7th Day	10th Day	Residue	Raw Material
Aspartic acid	13.39	10.68	12.58	0.73	6.75	86.66
Glutamic Acid	0	2.62	3.02	14.25	11.91	0
Asparagine	0.9	0	0	0	0	0
Serine	0.79	0	0	1.52	0	0.99
Glutamine	0	0	0	0	0	0
Histidine	10.92	4.483	5.881	1.99	0	6.06
Glycine	0	1.39	2.12	2.15	0	0
Threonine	168.51	174.37	280.96	0	177.24	218.19
Arginine	13.46	8.09	7.06	6.01	6.29	15.9
Alanine	0	0.76	1.04	1.63	323.91	189.85
Tyrosine	11.62	2.52	2.26	1.64	0	0
Cysteine	2.76	5.5	6.33	6.32	3.58	3.63
Valine	4.74	5	4.51	2.63	2.46	4.69
Methionine	83.97	53.39	57.08	56.07	95.18	93.73
Tryptophan	3.28	8.25	7.42	4.45	7.59	3.786
Phenylalanine	1.93	5.46	4.08	6.37	2.98	1.28
Isoleucine	8.63	13.56	19.75	17.61	18.56	9.57
Leucine	35.62	204.23	208.88	183.97	14.93	37.05
Lysine	2.95	7.5	6.56	1.32	0	0.86
Hydroxyproline	28.81	2.83	3.15	1.8	3.56	33.93
Proline	26.17	8.48	10.88	15.78	11.83	24.63
Total amt of AA	418.45	519.11	643.56	326.24	686.77	730.806

helps preservation effect. Fish protein hydrolysate using proteolytic enzyme such as papain helps to break down organic molecules like polypeptides, which is made of amino acids. Papain (*Carica papaya L.*) are naturally found in papaya and the unique structure of papain valuable for different purposes. Silver belly (*Liognatus splendens*) were more abundant in the area of coastal region of Rameswaram and are the only predominate by catch fish used in diets as significant proportions around 10-20% of dry weight without de-oiling. Silver belly accounts high quality protein content ranges 57.71% in commercial available fishmeal. Fat and protein content of silver belly accounts 2.9% and 16.5% studies on the effect of fish protein hydrolysate using enzyme and probiotics were made for the final product of liquid fish protein hydrolysate in various concentrations on seed germination. Past reports have recommended that cell reinforcement phenolic mixes may assume a job in advancing pea seedling improvement 17,43]. In present study, production of novel dry fish hydrolysate from silver belly using enzyme and bacteria were well demonstrated and their efficacy for plant growth promoters on seed germination carried out. Therefore the use of dry fish protein hydrolysate with 0.5% concentration as optimum to make good plant growth promoter as an efficient ecofriendly to the available conventional chemical fertilizers.

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